SOIL SCIENCE
RUTGERS COLLEGE

VOL. I NEW BRUNSWICK, N. J., JUNE, 1916 No. 6

PROTEIN DECOMPOSITION IN SOILS[1]

BY ELBERT C. LATHROP, *Biochemist in Soil Fertility Investigations, U. S. Department of Agriculture*

INTRODUCTION

From the standpoint of soil fertility the nitrogenous portion of the soil organic matter is of undoubted importance. Evidence, both direct and indirect, has been obtained which indicates that the larger portion of the nitrogenous matter of soils either is composed of proteins themselves or has been derived from proteins.

A number of investigators, working with soils from widely separated localities and of totally different origin and occupation, have shown that when the soils are treated by boiling with strong mineral acids the greater portion of the nitrogenous material goes into solution, whereas, before this treatment, it had been practically insoluble in the cold acids. It has been demonstrated, furthermore, that the acid solutions from the soils so treated gave, on analysis for the various forms of nitrogen, results very similar to those obtained by acid hydrolysis of proteins themselves. By means of isolation methods guanine (23), hypoxanthine, xanthine, arginine, and cytosine (46), decomposition products of nucleoproteins, have been obtained by partial hydrolysis of a soil with steam heat, though not found in the soil before heating. In addition, leucine and isoleucine have been obtained from Michigan peats (43) after hydrolyzing by boiling with strong acid. Such, in brief, is the indirect evidence for the occurrence of protein substances in soils.

The direct evidence of the protein nature of some of the nitrogenous portion of soil organic matter has been obtained almost entirely by Schreiner and his colleagues, Shorey, Lathrop and Walters, in their investigations on the composition of the organic matter of soils (48, 49, 45, 46, 53, 54, 55, 56, 60). These investigators have so far succeeded in isolating from soils the following nitrogenous compounds related to the proteins: proteoses and peptones; nucleic acids; the diamino acids, arginine, histidine and lysine; the pyrimidine base, cytosine; the purine bases, xanthine, hypixanthine and adenine; the base, choline, a decomposition product of phosphoproteins; and finally, creatinine, trimethylamine, tetracarbonimid and picoline carboxylic acid, most, if not all, possible secondary protein decomposition products. Very recently Potter and Snyder

[1] Received for publication May 13, 1916.

(41, 42), by the use of the Kober method (22), have been able to show that in some soils, at least, the amounts of free amino acids and peptides are very low.

These compounds, isolated from soils, together with many other decomposition products of proteins, have been investigated in regard to their action on plant growth (14,47,50), and it has been found that a large number of them are of direct value in plant nutrition, while others are toxic. A number of these can not only be used by the plants as a source of their nitrogen requirements but it has been shown that in water culture solutions, in the presence of nitrates, they are utilized by plants quite as readily as the nitrates themselves, thus indicating that these compounds may efficiently act as nitrate sparers.

Considered also from the standpoint of the energy expended by the plant in its metabolism, these compounds may play a very important part. Less energy, for example, is probably required in the synthesis of the plant proteins from compounds such as the amino acids or purine bases, which contain not only assimilable nitrogen, but carbon, hydrogen and oxygen as well, than in the synthesis of these same proteins from nitrate or ammonium salts alone, where the simpler components of the protein must in all probability be first synthesized and then these combined to complete the process. In the light of such a view it becomes of interest to observe that in the process of the decay of proteins in the soil they are split up first into the simpler compounds, such as peptides and amino acids, and that the greater portion of the ammonia is derived from these. These compounds are therefore presented to the plant at an earlier period in the decay of the protein than if the nitrogen compounds, in order to be utilized by the plant, had first to be changed into ammonia and then into nitrates.

Since in agricultural practice, protein material, of plant and of animal origin, is continually being added to the soil, it becomes necessary, from the practical and from the theoretical viewpoint as well, to obtain as accurate a picture as is possible of the changes which take place in proteins after they are introduced into the soil. This is especially important in connection with the interpretation of the action and availability of commercial organic fertilizers, barn-yard manure and green manures.

This investigation was therefore undertaken for the purpose of studying the changes taking place in protein material when added to an agricultural soil. Since ammonia formation is but one step in the process it becomes of interest to know from what portion of the protein molecule this ammonia is derived; to determine for how long a time the protein itself or the primary components of the protein can persist in the soil, and finally to get some insight into the nature of the protein compounds formed by the action of the microörganisms in their life processes.

TABLE I

COMPOSITION OF THE PROTEINS OF HORSE BLOOD AND CATTLE BLOOD

Results expressed in per cent

Amino acid	Globin of the oxy-hemoglobin of horse blood	Serum albumin of horse blood	Serum globin of horse blood	Nonpurified fibrin of cattle blood
Glycocoll	[1]0.00	[3]0.00	[5]3.52	[5]2.00
Alanine	[1]4.19	[3]2.68	[2]2.22	[5]3.60
Leucine	[1,9]29.04	[3]20.00	[2]18.70	[5]15.00
Phenylalanine ...	[1,9]4.24	[3]3.08	[2]3.84	[5]2.50
Proline	[1,9]2.34	[3]1.04	[2]2.76	[5]3.60
Glutamic acid	[1,9]1.73	[3]1.52	[2]2.20	[5]12.50
Aspartic acid	[1,9]4.43	[3]3.12	[2]2.54	[5]2.00
Cystine	[1]0.31	[5]2.53	[2]0.67	....
Serine	[1]0.56	[3]0.60	....	[5]0.80
Oxyproline	[1]1.04	....	....	....
Tyrosine	[1]1.33	[3]2.10	....	[5]3.50
Valine	....	....	....	[5]1.00
Lysine	[1]4.28	....	....	....
Arginine	[1]5.42	....	....	....
Histidine	[1]10.96	....	....	....
Tryptophane	[1]+	[3]+	....	....
Ammonia	[7]1.07	[6]1.01	[6]1.75	....
Cystein	....	[4]+	....	....

[1] E. Abderhalden (2). [2] E. Abderhalden (4). [3] E. Abderhalden (3). [4] G. Embden (9). [5] K. A. H. Mörner (37). [6] W. Hausmann (11). [7] W. Hausmann (12). [8] E. Abderhalden (5). [9] E. Abderhalden (6).

EXPERIMENTAL

Dried Blood

Dried blood which was chosen for this investigation, in addition to its suitability as a material high in protein matter, is a high grade nitrogenous fertilizer; that is, according to all tests it is of a high degree of availability for plant use and is used as a fertilizer in many sections of the United States and elsewhere, as such, and in mixed fertilizers. It is composed almost entirely of various animal proteins and the commercial product is of fairly constant composition. Abderhalden (1) reports figures on the composition of the blood of cattle, sheep, pigs, horses and goats, which show that a mixture of the blood of these animals should contain about 200 parts of solid matter for 1000 parts of blood. These solids consist of about 54 per cent hemoglobin and about 32 per cent albumin, or approximately 86 per cent proteins, exclusive of any nucleoproteins or nucleic acids which also are undoubtedly present. The products of the acid hydrolysis of the proteins of horse blood, globin of the hemoglobin, serum albumin and serum globin, and the non-purified fibrin of the blood of cattle, have been estimated in part by several investigators and the results so obtained are presented in Table I. The method used for the separation and estimation of the various amino acids is the esterification method proposed by E. Fischer, which is not strictly quantitative, involving losses

in the amounts of many of the amino acids, so that the figures obtained represent less than the actual amounts of the various hydrolysis products of these proteins.

The dried blood used in this investigation was purchased in the open market and contained 13.92 per cent of total nitrogen. Two 3-gm. samples of the dried blood were hydrolyzed by boiling with 60 c.c. of hydrochloric acid, sp. gr. 1.115 for 18 hours, after which time a positive biuret test could no longer be obtained, showing complete hydrolysis. The various forms of nitrogen in the hydrochloric extract were then estimated according to the nitrogen partition method proposed by Van Slyke (57), and the results so obtained are presented in Table II. Cystine nitrogen was not determined for the reason that it was thought that this determination would be of little value when studying the decomposition products of dried blood in soils; consequently any cystine nitrogen present is included with the nitrogen estimated as arginine, histidine and lysine.

The Soil Used

The soil was a Norfolk fine sandy loam taken from a cantaloup field near Raleigh, N. C. The soil was in a high state of cultivation and had received both mineral fertilizers and stable manure. It was found to contain 0.0301 per cent total nitrogen. The soil was passed through a 40 mesh sieve and dried *in vacuo*.

Forty parts of soil were mixed with about three parts of dried blood by sieving the two together repeatedly until samples taken from different parts of the mixture gave duplicate analyses for total nitrogen. The total nitrogen in the soil thus prepared was determined by the Kjeldahl-Gunning-Arnold method and was found to be 0.8945 per cent. The ammonia in the soil was determined by the vacuum distillation method recommended by the author (24) for the determination of ammonia in processed fertilizers and was found to be 0.0005 per cent. It should be stated that all analytical figures reported in this investigation are calculated on the oven-dried basis.

TABLE II

THE FORMS OF NITROGEN IN DRIED BLOOD AND IN THE EXPERIMENTAL SOIL
Results expressed in per cent of hydrolyzable nitrogen

Form of Nitrogen	Dried Blood	Experimental Soil
Amide nitrogen	6.854	7.008
Melanin nitrogen	2.600	4.767
Arginine nitrogen	7.517	7.601
Histidine nitrogen	12.523	12.366
Lysine nitrogen	11.517	10.093
Monoamino acid nitrogen	57.057	58.220
Non-amino nitrogen	1.479	0.312
Total	99.547	100.367

The soil was made up of 10 per cent moisture content, was kept in a 1-gallon stone-ware jar covered with perforated wrapping paper to exclude dust, and the decomposition was allowed to proceed at the temperature of the laboratory.

During the first 18 days the soil was kept at a constant moisture content of 10 per cent and was mixed several times by hand during that period to promote aeration. Later on, however, the soil was made up to 10 per cent moisture content every 5 to 8 days and on two occasions was allowed to dry out. At each addition of water to the soil it was dumped out of the jar and thoroughly mixed to promote aeration. The total length of the experiment was 240 days, during which time samples of the experimental soil were taken at the following intervals after it had been prepared: (1) 18 days, (2) 44 days, (3) 86 days, (4) 148 days, and (5) 240 days.

At the end of each period the soil was sampled, after a thorough mixing, by means of a brass tube which took a core of the soil from top to bottom. About eight borings were made at each sampling from different parts of the jar so that a fairly representative fraction of the soil was obtained. These different borings, amounting to about 300 gm. of moist soil, were then mixed well and placed in a small mason jar. All of the weighings for the analytical work were immediately made. In order to make sure that the sample in the mason jar was uniform, total nitrogen determinations were made on portions taken from the top and the bottom of the jar.

The dry mixture of soil and dried blood which was not used in preparing the experimental soil was placed in a glass stoppered bottle; total nitrogen and ammonia determinations made on samples of this taken from time to time showed that under such dry conditions no decomposition was taking place.

The amount of moisture in the various samples was determined by drying them in an oven for 2 hours at 103° C. Total nitrogen and ammonia determinations were made on samples of the experimental soil at the end of each period according to the methods already mentioned. Nitrates were not determined. One-hundred-gram samples of the soil at each sampling were subjected to hydrolysis by boiling with 200 c.c. of hydrochloric acid, sp. gr. 1.115, for 48 hours. The acid solution was filtered from the soil by suction and the soil was washed with boiling water until the washings became neutral in reaction. The combined acid filtrate and washings were concentrated at 40° C. under 10 mm. pressure to a thick syrup in order to get rid of most of the hydrochloric acid. The residue was taken up in hot water, the aqueous solution was filtered into a 250 c.c. volumetric flask and the filter thoroughly washed with hot water. After cooling, the solution was made up to the mark and total nitrogen

determinations were made on two 25-c.c. portions. The remainder of the respective solutions were subjected to the determination of the different forms of nitrogen, the details of the method as outlined by Van Slyke (57) being followed, excepting that the determination of cystine nitrogen was omitted.

The Analytical Results

By the use of the methods outlined the nitrogen was separated into the following: (1) total nitrogen in the soil, (2) total nitrogen in hydrochloric acid solution, (3) ammonia nitrogen in the soil, (4) ammonia nitrogen in the hydrochloric acid solution, (5) melanin nitrogen, (6) nitrogen precipitated by phosphotungstic acid, reported as arginine, histidine and lysine nitrogen, (7) nitrogen in the filtrate from the phosphotungstic acid precipitate, reported as monoamino acid nitrogen and non-amino nitrogen.

By subtracting the amount of ammonia nitrogen found in the soil (3) from the amount of ammonia nitrogen found in the hydrochloric acid extract (4) the amount of nitrogen in the soil in the form of the amide group in proteins or as acid amides may be obtained; this is reported as amide nitrogen. The amount of nitrogen in the soil in the form of proteins or protein decomposition products, with the exception of ammonia nitrogen, may be obtained by subtracting the amount of ammonia nitrogen in the soil (3) from the amount of total nitrogen in hydrochloric acid solution (2); this is reported as "hydrolyzable" nitrogen. The amount of nitrogen in all of the various fractions was determined by the Kjeldahl method, which does not include nitrate nitrogen unless large amounts of reducing substances are present. Such may be the case, however, with some of the Kjeldahl analyses and any nitrate nitrogen, therefore, included in a Kjeldahl determination would be reported as non-amino nitrogen.

In a recent article Van Slyke (58) states that the method which he has proposed for the partition of nitrogen was designed for use only with proteins not accompanied by other classes of substances, particularly nitrogenous substances, which would obviously falsify the interpretation of the results unless the behavior of the non-protein substances is so accurately known that corrections might be made. It should be clearly understood and constantly borne in mind that after the decomposition in the soil for any length of time of such complex organic compounds as those contained in dried blood, undoubtedly compounds other than proteins or the primary products of protein decomposition must make their appearance. Just what these compounds may be we can but conjecture at the present time, so that the results of the Van Slyke method when applied to the partition of the forms of nitrogen in soils, while reported as arginine nitrogen, histidine nitrogen, etc., can be considered as being only ap-

proximations for the amounts of these various forms of nitrogen actually present in the soil as proteins or as the primary products of protein decomposition. It should be clearly emphasized, however, that the method is of decided value, even under limiting circumstances, in attacking such a problem as the one at hand, since by the use of such a method it is possible to divide the nitrogenous compounds present in the soil into a number of classes which react towards the various reagents involved in the analytical procedure as though they were arginine nitrogen, histidine nitrogen, etc. The very fact that a given nitrogenous compound will, towards a given chemical reagent or a series of them, react like arginine, histidine, etc., establishes a chemical and possibly a biochemical relationship.

In regard to the nitrogen reported in this investigation as amide nitrogen it might be stated that it is difficult to conceive in the present state of our knowledge of any soil compounds other than the amide group of the various proteins, or the acid amides themselves, which would resist heating *in vacuo* with calcium hydroxide and subsequently split off ammonia on heating with hydrochloric acid.

The melanins are at present undefined and no significance can be attached to the figures obtained.

The nitrogen reported as monoamino nitrogen includes all nitrogenous compounds not precipitated by calcium hydroxide or not volatile in its presence *in vacuo*, not precipitated by phosphotungstic acid and containing a free amino group which will react with nitrous acid to produce free nitrogen.

The greatest inaccuracies occur in the diamino acid fraction and these are distributed between arginine, histidine and lysine nitrogen. This group includes all nitrogenous compounds which are precipitated by phosphotungstic acid, excepting the ammonia and melanin nitrogen which have been previously removed.

The nitrogen reported as non-amino nitrogen includes all nitrogenous compounds not accounted for in the above and may include a small amount of nitrogen present in the soil in the form of nitrates.

The results obtained by the methods outlined are presented in Tables IV and V.

Hydrolysis

The amount of dried blood added to the Norfolk fine sandy loam is far in excess of the amount ever added in good agricultural practice. However, this amount was found by experiment to be necessary in order to obtain accurate analytical results; furthermore, it seemed desirable to add enough dried blood protein to the soil to render the small amount of soil protein neglible, so that only the fertilizer nitrogen would be under observation. By reference to Table II, in which the results of the Van

Slyke method as applied to the mixture of blood and soil are reported, it will be observed that the figures obtained for the various forms of nitrogen correspond very closely to those obtained from the dried blood alone, except the figures for melanin and non-amino nitrogen, but the reason for this is not altogether clear.

Under natural conditions the changing of organic nitrogen into ammonia is the work of microörganisms in the soil. Müntz and Coudon (38), studying the ammonification in sterilized and unsterilized soil, showed that during two and one-half years there was no ammonia formation in the sterilized soil, while the unsterilized soil in 67 days produced from 41 to 110 mg. of ammonia per 100 gm. of soil. Ammonia formation during the bacterial or mold decomposition of protein materials is an evidence of chemical changes in the protein molecule and the total amount of ammonia formed during the entire decomposition process may in a general way, perhaps, be considered an index of the extent of these changes. The interest in the present investigation centers in establishing the actual chemical origin of this ammonia, the portions of the protein molecule from which it is split by the soil organisms and thus elucidating the chemical changes involved in the disappearance of this type of organic matter from soils. It is obvious that for a full appreciation and understanding of these biochemical changes which occur during the decay of proteins in the soil, a knowledge of the molecular structure of the proteins and of the mechanism of microörganismal action is very essential.

The synthetic researches of Emil Fischer and his pupils, begun in 1901, on the structure of the protein molecule, prove the accuracy of Hofmeister's (13) view that the acid amide combination of the amino acids is the principal one in the protein molecule, according to the general structure:

$$NH_2—CHR.CO—(NH.CHR.CO)x—NH.CHR.COOH.$$

The chemical nature of an albumin is apparently partly determined by the quantitative relationships of the different amino acids and partly by the arrangement of these amino acids in the protein molecule. Two points regarding the constitution of the protein molecule have been fairly conclusively established, which have a direct bearing on this study. A small portion of the total nitrogen of the protein molecule is liberated as ammonia on hydrolysis; this points to the presence of linkings in the form of acid amide ($—CO—NH_2$) combinations. From a study of the amounts of ammonia formed by the hydrolysis of a large number of proteins by acids and the amounts of ammonia formed by heating these proteins with a solution of sodium hydroxide, Osborne, Leavenworth and Brautlecht (40) conclude that it is highly probable that the ammonia results from an amide union in the protein molecule. Van Slyke and Birchard (59) from a study of the action of certain proteins towards

nitrous acid, conclude that one of the two amino groups of lysine, the ω-group, exists free in the protein molecule. This group represents within, at most, a fraction of a percentage of the protein nitrogen, the entire amount of free amino nitrogen determinable in the native proteins by the nitrous acid method. The α-groups, which constitute the remaining and greater part of the free amino nitrogen found after complete hydrolysis, are in the intact protein molecule practically all condensed into peptide linkings. With primary albumoses, the first decomposition products of proteins, the relations are different; the free amino nitrogen in hetero- and protoalbumoses exceeds half of the lysine nitrogen by 3.00 and 4.80 per cent of the total nitrogen respectively, indicating that an appreciable portion of the α-amino groups of other amino acids is uncovered even in primary digestion products.

Hydrolysis of the protein molecule by means of various chemical reagents and enzymes results in the introduction of water into the molecule at various places with the appearance of albumoses, peptones, polypeptides, amino acids and ammonia, the amounts and nature of the products depending on the nature of the protein and the specific reagents used. The final products of acid hydrolysis are the amino acids and ammonia, while with pepsin no amino acids are said to be formed, the splitting resulting in the formation of albumoses, peptones, peptides and ammonia. Trypsin differs from pepsin in that, although it cannot attack all proteins, requiring in some instances the action of pepsin first, it splits the molecule more deeply, with the formation of amino acids, together with many of the products formed by peptic digestion.

In regard to the decomposition of proteins by microörganisms, numerous investigations have been made and the following general conclusions may be drawn from them. There is little reason to suppose that the action of microörganisms is other than that of the enzymes which they produce. Kruse states that bacterial proteolytic enzemes resemble both pepsin and trypsin in the nature of their action but are different from either. The degradation of proteins by microörganisms proceeds along the same general lines as that produced by proteolytic enzymes and acids but the process does not stop with hydrolytic cleavage, a deeper change taking place with the formation of large amounts of ammonia and carbon dioxide, together with amines, fatty acids, alcohols, aldehydes, hydrogen sulfide, methane, phenol, skatol, indol, etc.

Ammonia Production

Two sorts of splitting by which ammonia is formed deserve consideration: first, the production of ammonia by direct hydrolysis of the proteins, with the consequent destruction of the amide group, (—CO—NH_2) contained in the proteins; second, the formation of ammonia from other portions of the protein molecule. For instance, if ammonia were formed

by the splitting off of only the amide group from the proteins of the dried blood, then, the total amount of ammonia produced during the entire decomposition would amount to about 7.0 per cent of the total nitrogen of the fertilizer. However, as may be seen from the results presented in Table III, the total amount of ammonia nitrogen produced during the 240-day period in the soil represents about 79.0 per cent of the total nitrogen originally present in the dried blood. It is evident, therefore, that ammonia has been formed from other fractions of the protein molecule besides that containing the amide linking.

In regard to the action of microörganisms on amino acids it may be stated that the chemical changes involved depend largely upon the character of the organisms, the condition of growth especially with regard to the presence or absence of oxygen, and the available sources of nutrient other than amino acids. In general, it may be said that anaerobic bacteria are prone to reduce α-amino acids with the formation of fatty acids and the liberation of ammonia, (equation I). Aerobic bacteria more frequently oxidize the α-amino acids to a fatty acid containing one less carbon atom, carbon dioxide and ammonia being set free (equation II). Yeasts have been shown by Ehrlich to convert amino acids into alcohols, carbon dioxide and ammonia (equation III), the net result of this reaction indicating neither oxidation or reduction but simple hydrolysis with carbon dioxide liberation. Another type of reaction (equation IV) very commonly brought about by bacteria involves the liberation from amino acids of carbon dioxide but not ammonia; it is in this manner that amines may be formed. The type reactions involved in these various changes may be represented as follows (8):

I. $R.CH_2.CHNH_2.COOH + H_2 \longrightarrow R.CH_2.CH_2.COOH. + NH_3$ (Reduction).
II. $R.CH_2.CHNH_2.COOH + O_2 \longrightarrow R.CH_2.COOH + NH_3 + CO_2$ (Oxidation).
III. $R.CH_2.CHNH_2.COOH + H_2O \longrightarrow R.CH_2.CH_2OH + NH_3 + CO_2$ (Hydrolysis).
IV. $R.CH_2.CHNH_2.COOH \longrightarrow R.CH_2.CH_2NH_2 + CO_2$ (Amine formation).

A combination of a number of these reactions may be effected by a single organism and different results may often be obtained using the same organism under varying conditions.

The investigations concerned with the process of ammonification in the soil cover a large number of years and a résumé of this work is not deemed essential. However, among the investigations more recently conducted may be mentioned those by Löhnis (33, 34, 35), J. G. Lipman and his co-workers (27, 28, 29, 30, 31), C. B. Lipman and P. S. Burgess (26), P. E. Brown (7), W. P. Kelly (20), W. G. Sackett (44) and H. C. McLean and G. W. Wilson (36). From the results obtained by these investigators and others it is apparent that there are many factors which are involved in the process of ammonification of organic nitrogen. Some of these factors are: soil moisture, aeration of the soil, the mineral salts present, the physical and chemical nature of the nitrogenous matter, the

amount of organic matter present and the depth of the layer through which this is distributed, and the type and number of the organisms at work in the soil.

Assuming that ammonification of protein material in soils must precede nitrification and denitrification and that all loss of nitrogen in this investigation is due to ammonia evaporation, nitrification or denitrification, and that free nitrogen is not split off from compounds other than nitrates or nitrites, then it is possible to arrive at the amount of ammonia formation in the soil during each period of time. It should be stated that this is ammonia formed exclusive of ammonia assimilated, there being no way in which ammonia assimilation could be accurately determined in this experiment.

This ammonia formation may be calculated from the following equations:

Total N—NH_3 nitrogen in the original soil=A.

Total N—NH_3 nitrogen in soil at end of each period=B.

Then A—B=X, or ammonia formation during the period.

$\frac{X}{A}$ =per cent of nitrogen changed to ammonia during the period.

TABLE III

PER CENT OF TOTAL NITROGEN IN THE SOIL AMMONIFIED AT THE END OF EACH PERIOD OF SAMPLING

Time from the beginning of the experiment	Per cent of total nitrogen
18 days	18.72
44 days	54.03
86 days	72.66
148 days	78.13
240 days	78.92

Table III, in which are presented the results obtained by the use of the above formula, shows that about 79 per cent of the nitrogen of the dried blood was converted to ammonia in 240 days. At the end of 86 days, less than half the total length of the experiment, about 73 per cent of the nitrogen of the dried blood had been changed into ammonia, showing that not only was the amount of ammonia formed during the remaining 154 days very small but that the rate of ammonification of the nitrogenous matter of the soil was greatly reduced, being about 10 per cent of the rate during the first period of 18 days.

The Results of the Van Slyke Analysis

By comparing the results obtained by the Van Slyke analysis of each soil sample during the experiment with the results obtained on the original soil the amounts of gain or loss in the eight different forms of nitrogen can be arrived at. It is thus possible to determine how rapidly any

particular form of nitrogen compound disappeared from the soil in the course of the decomposition and, further, to determine the relative amounts of nitrogen in these fractions in respect to the total amount of nitrogen present in the soil at the end of any period. When an increase in any particular form of nitrogen over the amount present in the soil during the previous period is observed it is not possible in all cases to state the compound in which this nitrogen existed, but when a certain form of nitrogen shows a loss during a period it is an absolute indication that that particular kind of nitrogen was disappearing or had disappeared from the soil, although the rate could not be determined. The results obtained by these analyses are presented in Table IV, in which the amounts of nitrogen in the various fractions are reported in per cent of the hydrolyzable nitrogen of the original soil. The results were all obtained by direct analysis, except in the fifth period when the melanin nitrogen was obtained by difference.

TABLE IV

THE FORMS OF NITROGEN IN THE SOIL AT THE END OF EACH PERIOD

Hydrolyzable nitrogen in the original soil = 100

Forms of nitrogen	Original soil	Time in days from the beginning of the experiment				
		18	44	86	148	240
Amide nitrogen	7.008	7.515	6.025	5.429	3.454	3.222
Melanin nitrogen	4.767	5.080	4.374	2.276	1.391	1.698
Arginine nitrogen	7.601	5.162	3.041	1.857	1.342	1.395
Histidine nitrogen	12.366	12.975	5.547	2.912	2.382	2.010
Lysine Nitrogen	10.093	7.610	1.110	0.429	0.528	0.972
Monoamino acid nitrogen	58.220	40.493	18.612	8.970	7.938	7.187
Non-amino nitrogen	0.312	1.120	1.675	2.191	0.738	0.297
Hydrolyzable nitrogen	100.000	79.660	40.598	24.070	17.740	16.741

The figures presented in Table V represent the relative amounts of the various forms of nitrogen in percentages of the hydrolyzable nitrogen of the soil present at the end of each period. From this table the fluctuating composition of the hydrolyzable nitrogen of the soil may be followed and the final composition of the hydrolyzable nitrogenous matter of the soil may be established.

TABLE V

THE FORMS OF NITROGEN IN THE SOIL AT THE END OF EACH PERIOD

Hydrolyzable nitrogen in the soil at the end of each sampling period = 100

Forms of nitrogen	Original soil	Time in days from the beginning of the experiment				
		18	44	86	148	240
Amide nitrogen	7.008	9.555	14.840	22.556	19.471	19.246
Melanin nitrogen	4.767	6.375	10.773	9.453	7.657	10.910
Arginine nitrogen	7.601	6.477	7.491	7.717	7.567	8.333
Histidine nitrogen	12.366	16.276	13.663	12.099	13.421	12.006
Lysine nitrogen	10.093	9.550	2.710	1.784	2.979	5.809
Monoamino acid nitrogen	58.220	50.812	45.847	37.264	44.745	42.922
Non-amino nitrogen	0.312	1.410	4.125	9.102	4.160	1.774
Hydrolyzable nitrogen	100.000	100.000	100.000	100.000	100.000	100.000

The figures presented in Table VI show the amounts of loss of nitrogen in each form in the soil at the end of each sampling period. The amount of loss is stated in percentages of the largest amount of any form of nitrogen in the soil at any time; for example, in the case of the amide nitrogen the amount is largest at the end of 18 days, and this figure is taken as 100. In this table the word "gain" indicates an increase in the amount of nitrogen over that present at the end of the preceding period.

TABLE VI

THE PERCENTAGE LOSS OF the VARIOUS FORMS OF NITROGEN IN THE SOIL AT THE END OF EACH SAMPLING PERIOD

The largest amount of nitrogen in the soil = 100

Form of nitrogen	Time in days from the beginning of the experiment				
	18	44	86	148	240
Amide nitrogen	Gain	20	28	55	57
Arginine nitrogen	31	60	76	83	Gain
Histidine nitrogen	Gain	58	80	82	83
Lysin nitrogen	24	89	96	Gain	Gain
Monamino acid nitrogen	31	67	84	86	89
Hydrolyzable nitrogen	20	59	76	82	83

Hydrolyzable Nitrogen

During the 240-day decomposition of the dried blood in the soil a loss of 83 per cent of the total hydrolyzable nitrogen took place. At the end of 86 days the loss was 76 per cent, showing that during the latter and longer portion of the decomposition experiment the amount of hydrolyzable nitrogen which vanished from the soil was extremely small.

Monoamino Acid Nitrogen

During the experiment the monoamino acid nitrogen dimished from 58 to 7 per cent, or a loss of 89 per cent of the total monoamino acids originally present in the proteins. At the end of 18 days, 31 per cent of this form of nitrogen had vanished, while during the same time only 20 per cent of the hydrolyzable nitrogen was lost. Since the monoamino acids contain more than half of the total hydrolyzable nitrogen, it appears that the relative loss from each would be about the same. The fact that there is a difference of about 11 per cent between the losses from these fractions leads to the supposition that nitrogen split off from the monoamino acids has been assimilated by the microörganisms in the formation of their protoplasm.

It may be stated in this connection that it has been found by the few investigations concerned with the chemical nature of the protoplasm of microörganisms that this protoplasm is composed to a greater or less extent of proteins depending somewhat upon the nature of the media upon which the organisms have developed. Regarding the general nature of the proteins of bacteria and mold protoplasm a number of investigations

have been conducted, but aside from the isolation of some protein-like substances and some nucleic acids from this sort of protoplasm, together with the isolation of some amino acids from the hydrolysis products of these substances, not much is actually known concerning the real chemical composition and structure. In regard to the nitrogen compounds which are present in the protoplasm of soil organisms, Omelianski and Sieber (39) report that the bodies of *Azotobacter chroococum* contain about 13 per cent of nitrogen, which, by analysis according to the Van Slyke method, they found to be distributed as follows: amide nitrogen 9.6, melanin nitrogen 3.5, arginine nitrogen 10.13, histidine nitrogen 1.64, lysine nitrogen 14.60, monoamino acid nitrogen 55.40, and non-amino nitrogen 4.86 per cent, respectively, of the total hydrolyzable nitrogen. The composition of the protein of other organisms would probably differ.

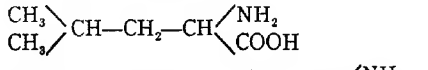
In Table V it will be observed that the proportion of the monoamino acids present in the soil at the various times of sampling fluctuates. The lowest figure is 37 per cent at the end of 86 days.

Lysine Nitrogen

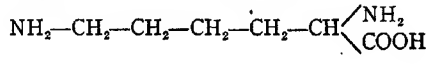
The analytical results show that lysine disappears from the soil quite rapidly. At the end of 44 days, 89 per cent of the lysine originally present in the proteins has been decomposed, and at the end of 86 days, 96 per cent. During the remaining and longer part of the decomposition period there is a continual gain in lysine nitrogen, indicating that synthetic processes are at work.

The gain in lysine nitrogen, after the original had practically vanished from the soil, is to be attributed to the action of the microörganisms in synthesizing some compound or compounds which give the analytical reactions for lysine. That this increase is due entirely to lysine cannot be stated, but lysine no doubt makes up a part of the gain observed.

It will be noted from Table VI that the two fractions which show the greatest amount of loss during the experiment are lysine nitrogen and monoamino acid nitrogen. It is not surprising that these two show the greatest loss when their chemical composition is considered. The monoamino acids are straight chain acids with the amino group in the alpha position to the carboxyl group. Lysine, a diamino acid, is also a straight chain acid containing two amino groups, one in the alpha position to the carboxyl group and one at the extreme end of the chain from the carboxyl group, or in the omega position. The relationship between lysine and the amino acids may be clearly shown by presenting the structural formulas for lysine and for leucine, for example:



Leucine.



Lysine.

However, it is observed that the lysine vanishes more quickly from the soil than the monoamino acids. This may be due to the fact that the ω-amino group of the lysine exists free in the molecule of the native proteins which occur in the dried blood. Under such conditions this group is subject to deaminization by the action of the microörganisms before hydrolysis takes place, while in the case of the monoamino acids hydrolysis must precede deaminization since these acids are linked in the protein molecule in anhydride structure. Furthermore, if the omega group be split off from the lysine while it is still a constituent part of the protein molecule it is changed into an amino acid with but one amino group and would be determined analytically as monoamino acid nitrogen.

From Table V it will be observed that there are very marked fluctuations in the proportions of lysine nitrogen in the soil at the end of each period. The lowest amount occurs in the soil at the end of 86 days, which was the low point for monoamino acids. The final amount is about half that originally present in the dried blood.

Histidine Nitrogen

At the end of 18 days the histidine nitrogen showed a gain. Although the compounds which cause this increase cannot be arrived at, it is possible that they are, in part at least, the purine and pyrimidine bases, which by the analytical methods would be classed as histidine nitrogen. It is well known that the protoplasm of microörganisms is made up of considerable amounts of nucleoproteins and nucleic acid, which on hydrolysis would yield the purines and pyrimidines.

At the end of 44 days 60 per cent of the histidine nitrogen had disappeared; at the end of 86 days, 80 per cent, and after 240 days, 83 per cent.

The proportion of the histidine nitrogen in the soil at the various times of sampling is about constant, with the exception of the 18-day sample.

Arginine Nitrogen

After 18 days 31 per cent of the arginine had vanished from the soil, while at the end of 148 days 83 per cent had gone. From the 148th to the 240th day, a period of 92 days, a gain in arginine nitrogen was observed. This may be due to nitrogen in the form of arginine, or nitrogen in the form of compounds which give the analytical reactions for arginine. It is nitrogen in organic compounds formed by the action of microörganisms, and is possibly in the form of proteins.

The relative amount of arginine nitrogen showed little fluctuation throughout the experiment and was a little greater at the end of the experiment.

Amide Nitrogen

The analysis of the figures for amide nitrogen brings out some interesting points. After 18 days there was an increase in amide nitrogen. It may be safely assumed that the compounds which this increase represents are acid amides, formed by the action of the microörganisms, existing in the soil either free or combined in the molecule of some new proteins contained in the protoplasm of organisms. That there was actually an increase in this form of nitrogen after 18 days was, however, unexpected, since it is well known that microörganisms, when grown in solutions of acid amides can use them for the building up of their protoplasm, and, furthermore, Jodidi (18) has shown that acid amides are very easily and quickly ammonofied when placed in an agricultural soil. It was therefore expected before the results were obtained that the amide nitrogen would be one of the forms which would most quickly disappear from the soil. From Tables IV and VI it will be observed that this fraction disappears least completely and most slowly.

The question arose as to whether the soil used was capable of ammonifying acid amides. Consequently, 1 gm. of pure asparagine, one of the two acid amides considered to be present in the protein molecule, was added to 100 gm. of the air-dried Norfolk fine sandy loam to which no dried blood had been added. The soil was made to about a 10 per cent moisture content and allowed to stand for 4 days. On analysis for ammonia it was found that the soil had converted 73.4 mg. of asparagine nitrogen into ammonia nitrogen in this time, or in other words, the soil in 4 days had ammonified 39.3 per cent of the total asparagine nitrogen. This indicates that free acid amides in the soil would have been to a very large extent converted into ammonia during the 18 days of the experiment, and points unquestionably to the fact that the increase in this form of nitrogen is due to the synthetic action of the microörganisms in the building up of their own protoplasm.

After establishing the fact that the soil was capable of ammonifying acid amides it was decided to ascertain, if possible, if at any time previous to the first sampling period there occurred a decrease in amide nitrogen and at what time the increase in this form of nitrogen was first observable by the analytical methods. For this purpose some of the original mixture of soil and dried blood which had been shown to have undergone no change during storage, was taken and kept at a 10 per cent moisture content. Samples of this soil were taken at short intervals and analyzed for their content of free ammonia in the soil and ammonia in the hydrochloric acid extracts after hydrolysis. From these data it was possible to arrive at the amounts of amide nitrogen in the soil at the end of each sampling period. The results so obtained, together with the results already obtained upon amide nitrogen, are presented in Table VII.

TABLE VII
AMIDE NITROGEN IN THE SOIL AT VARIOUS PERIODS

Time from the beginning of the experiment	Mg. of amide nitrogen per 100 gm. of oven-dried soil	Amide nitrogen expressed in percentages of hydrolizable nitrogen in original soil
Original soil	57.14	7.008
2 days	59.77	7.329
3 days	60.15	7.363
5 days	8.75	1.606
6 days	34.31	4.207
7 days	38.42	4.628
8 days	60.42	7.408
13 days	57.48	7.020
18 days	61.39	7.515
20 days	60.72	7.110
44 days	49.13	6.025
86 days	44.38	5.429
148 days	28.27	3.454
240 days	26.38	3.222

The results show that during the second and third day there has been a slight increase in amide nitrogen. This must be considered as being due to the formation of protein material by the microörganisms in the form of their protoplasm, and since there has probably been little hydrolysis of the dried blood proteins at this time, the nitrogen necessary for this synthesis may have been derived from the free amino groups of the lysine of the native proteins of the dried blood, or from the free amino groups of lysine or other amino acids in albumoses which are also possibly present in the dried blood. On the fifth day the amide nitrogen had fallen from 60.15 mg. at the end of the third day tc 8.75 mg., a loss of about 70 per cent of this form of nitrogen. Since the number of organisms in the soil is constantly increasing a portion of the amide nitrogen present in the soil at this time must be present in the form of proteins constituting the protoplasm of these organisms. It would appear, therefore, that practically all of the amide nitrogen of the dried blood proteins has been split off in 5 days, signifying a deep hydrolysis of these proteins.

The amide nitrogen increased from the fifth to the sixth day from 8.75 to 34.31 mg., the seventh day shows a further increase and on the eighth day the amount of amide nitrogen was about that present in the soil at the end of the third day; from the eighth to the twentieth day the amount of amide nitrogen, aside from small fluctuations, remained almost constant. This increase must be considered as being due to the synthetic action of the microörganisms in the formation of their protoplasmic proteins. In view of the fact that these proteins, in amount, must be much smaller than the proteins of the dried blood, but that the amide nitrogen content of the soil is even greater than the content of the original soil, it

would appear that the proteins formed by the microörganisms must be relatively rich in amide linkages.

The amide nitrogen of the soil during the time covered from the end of the first period of 18 days to the end of the experiment, 222 days, shows a loss of 57 per cent. This loss is by far smaller than any of the other forms of nitrogen. Since the amide nitrogen was practically all destroyed at the end of the fifth day and then amide nitrogen was synthesized, it would appear that the amide nitrogen built up by the action of microörganisms exists in proteins which are more resistant to the action of the microörganisms than were the proteins of the dried blood.

The figures in Table V show that the proportions of amide nitrogen in the soil increase up to the 86th day, when 22 per cent is reached as compared with 7 per cent in the original soil. The amount then drops off to 19 per cent.

In this connection it is extremely significant that the results obtained by Shorey (51), Lathrop and Brown (25), Jodidi (15, 16, 17), Kelly (20), and Potter and Snyder (41, 42) on hydrolyzing the nitrogenous compounds of soil and peats from this country and Hawaii, show amounts of amide nitrogen in the soils uniformly higher than are found by acid hydrolysis of animal or of vegetable proteins. These figures range between 16 and 30 per cent of the total hydrolyzable nitrogen of the soils. It has further been shown that some of these soils readily ammonify acid amides, indicating that the amide nitrogen exists in protein complexes and not as free acid amides. In the light of the present investigation these various analytical results seem to point to the presence in soils of considerable amounts of microörganismal proteins.

Melanin and Non-amino Nitrogen

Owing to the fact that the non-amino nitrogen varies so much throughout the experiment and that the melanins are so little understood, these two forms of nitrogen cannot be profitably discussed.

The results of this investigation are not in strict accord with those recently obtained by Kelly (21), who studied the decomposition of various sorts of organic matter in Hawaiian soils. He determined the amide, basic, and nonbasic nitrogen in casein, dried blood, soybean cake, cotton-seed meal, linseed meal, cocoanut meal, globulin from cotton seed, and zein from maize, before and after the action of bacteria on these compounds in quartz sand to which a soil infusion has been added previous to incubation. His experiments covered from 3 to 8 days' decomposition and the amounts of organic matter used were about one-fourth the amount used in this investigation. He found that, with the exception of linseed meal and zein, the diamino nitrogen was converted into ammonia more rapidly than any other form of nitrogen. In the present investiga-

tion the conversion of the diamino nitrogen, arginine, histidine and lysine, into ammonia in 240 days amounts to 87 per cent, and the monoamino nitrogen 89 per cent. The causes for the differences in the results are probably not only the different experimental conditions but also a difference in the microörganismal flora, producing different types of the decomposition.

Proteins in the soil at the end of the experiment

From the results of the Van Slyke analysis evidence has been found to indicate that there is a formation of protein taking place in the soil in the course of the decomposition of protein materials, and that perhaps this new protein is somewhat resistant to decomposition. In order to determine whether or not soluble proteins are present in the soil after the decomposition had been proceeding 240 days the portion of the soil which remained was extracted with distilled water for several hours. The solution was then decanted from the soil and filtered. Tests for proteins or protein-like substances in the solution showed that such compounds had not been extracted by distilled water.

The soil was then treated with a 1 per cent solution of sodium hydroxide for 24 hours and this alkaline solution siphoned off from the soil. This solution was acidified with sulfuric acid and was filtered. To this acid filtrate 20 per cent phosphotungstic acid solution was added until precipitation had ceased, and after allowing the solution to stand for several hours until the precipitate had settled the precipitate was filtered off by suction and thoroughly washed with water acidulated with sulfuric acid. The phosphotungstic acid precipitate was suspended in cold distilled water and treated with an excess of barium hydroxide solution in order to free the protein material from the phosphotungstic and sulfuric acids. The excess of barium in the filtrate from the precipitate so formed was removed by carbon dioxide and the barium carbonate was filtered off. The solution was made just acid with dilute sulfuric acid and was boiled for a minute with a little barium carbonate and then filtered. A light straw-colored, turbid solution was obtained, which behaved in general like solutions of protein material. This solution was tested for the presence of proteins or protein-like substances. It gave precipitates with phosphotungstic, phosphomolybdic, tannic and picric acids, with mercuric chloride, silver nitrate, and copper acetate. The following tests for proteins were positive: Millon's reaction; Biuret test (reddish violet); Spiegler's ring test (weak); Robert's ring test (weak); Hopkins-Cole reaction, and Liebermann's reaction. Acetic acid and potassium ferrocyannide solution when added to the soil extract did not produce a precipitate, but a precipitate was formed when a solution of sodium chloride containing acetic acid was added. The protein material could be salted out by solid sodium chloride and by ammonium sulfate. A precipitate was formed on the addition of sufficient alcohol to make a 50 per cent

alcoholic solution, and the filtrate from this precipitate when treated with a large amount of absolute alcohol formed a further precipitate. A distinct cloudiness was formed in the solution on the addition of a half-saturated solution of ammonium sulfate. By these reactions the presence of proteins or protein-like substances in the soil is established. The exact class to which this protein material belongs could not be determined except by a more extended investigation. This established the fact that after a 240-day decomposition of dried blood in the soil, proteins, or protein-like complexes, not extractable by distilled water but soluble in dilute alkaline solution, were present in the soil. Whether they were proteins from the bodies of microörganisms in the soil, or whether they were residues from the dried blood which had until that time resisted decomposition by the microörganisms of the soil cannot be stated.

Of interest in this connection is the fact that Walters (60) has recently reported the isolation from a field soil of protein-like complexes which gave reactions for proteoses and peptones, bodies similar in nature and reaction to the compound here reported.

No attempt was made to isolate free amino acids from the soil after the decomposition period, since the quantities of soil were too small.

Summary

The ammonification of the dried blood in the soil during the first 86 days was very rapid, after which time the amount of ammonia produced and the rate of ammonification decreased markedly until the end of the experiment. At the end of the experiment the rate of transformation of hydrolyzable nitrogen into ammonia nitrogen in the soil was but about 10 per cent of the rate observed after the decomposition had been proceeding for 18 days. During the 240 days of the experiment 79 per cent or more of the nitrogen of the dried blood proteins was converted into ammonia nitrogen.

The ammonia produced during the decomposition of the dried blood was derived from (1) the hydrolytic cleavage of the proteins of the dried blood, as evidenced by the rapid vanishing of the amide compounds from the soil during the first five days of the experiment; and (2) from the decomposition by the microörganisms of the products resulting from the hydrolytic cleavage of the proteins. Some of the ammonia produced during the first two or three days, when the hydrolysis of the proteins does not seem to have been very extended, may possibly have been due to the deaminization of the ω-amino group of the lysine in the native proteins of the dried blood. With the exception of the amide compounds lysine seems to have disappeared most rapidly and completely from the soil. The monoamino acids contributed about 89 per cent of their nitrogen to the formation of ammonia, and arginine and histidine each contributed about 83 per cent.

An analysis of the figures obtained by the Van Slyke method points to the generation of new protein materials in the soil. This is indicated by (1) the unequal loss of monoamino acids and hydrolyzable nitrogen from the soil during the early stages, (2) by an increase in amide nitrogen during the early stages, (3) by an increase in histidine nitrogen during the early stages, (4) by an increase in arginine nitrogen during the later stages, and (5) by an increase in lysine nitrogen during the later stages.

This new form of protein seems to be more resistant to the action of the microörganisms than were the proteins of the dried blood, since the amide compounds of the dried blood vanished very largely from the soil in 5 days but the amide compounds produced in the soil decreased only to the extent of 57 per cent during the remaining 222 days of the experiment, and also since the lysine of the dried blood almost entirely disappeared from the soils during the first 86 days of the experiment, but during the last 154 days of the experiment a continual increase in this form of nitrogen was observed.

Protein-like substances, non-extractable by distilled water but extractable by 1 per cent sodium hydroxide solution, were isolated from the soil after the dried blood had decomposed for 240 days. Whether these were residues from the dried blood which had until this time resisted decomposition by the microörganisms or were proteins produced by the microörganisms cannot be stated.

LITERATURE CITED

(1) ABDERHALDEN, E.
1898. Zur quantitative vergleichenden Analyse des Blutes. *In* Ztschr. Physiol. Chem., Bd. 25, p. 65-115.

(2) ABDERHALDEN, E.
1903. Hydrolyse des krystallirten Oxyhämoglobins aus Pferdeblut. *In* Ztschr. Physiol. Chem., Bd. 37, p. 484-494.

(3) ABDERHALDEN, E.
1903. Hydrolyse des krystallirten Serum-Albumins aus Pferdeblut. *In* Ztschr. Physiol. Chem., Bd. 37, p. 494-498.

(4) ABDERHALDEN, E.
1905. Abbau und Aufbau der Eiweisskörper im tierischen Organismus. *In* Ztschr. Physiol. Chem., Bd. 44, p. 17-52.

(5) ABDERHALDEN, E., and BAUMANN, L.
1907. Die Monoaminosäuren des krystallirten Oxyhämoglobins aus Hundeblut. *In* Ztschr. Physiol. Chem., Bd. 51, p. 397-403.

(6) ABDERHALDEN, E., and VOJTINOVICI, A.
1907. Weitere Beiträge zur Kenntnis der Zusammensetzung der Proteine. *In* Ztschr. Physiol. Chem., Bd. 52, p. 368-374.

(7) BROWN, P. E.
1913. Bacteriological Studies of field soils III. Iowa Agr. Exp. Sta., Research Bul. 13.

(8) DAKEN, H. D.
1912. Oxidations and reductions in the animal body. Biochemical monograph, VIII + 135 p., London, New York and Bombay.

(9) EMBDEN, G.
1901. Ueber den Nachweis von Cystin und Cystein unter den Spaltungsprodukten der Eiweisskörper. *In* Ztschr. Physiol. Chem., Bd. 32, p. 94-103.

(10) FISCHER, E., and ABDERHALDEN, E.
1903. Ueber die Verdauung einiger Eiweisskörper durch Pankreasfermente. *In* Ztschr. Physiol. Chem., Bd. 39, p. 81-94.

(11) HAUSMANN, W.
1899. Ueber die Vertheilung des Stickstoffs im Eiweissmolekül. *In* Ztschr. Physiol. Chem., Bd. 27, p. 95-108.

(12) HAUSMANN, W.
1900. Ueber die Vertheilung des Stickstoffs im Eiweissmolekül. *In* Ztschr. Physiol. Chem., Bd. 29, p. 136-145.

(13) HOFMEISTER, F.
1902. Ueber Bau und Gruppierung der Eiweisskörper. *In* Ergeb. Physiol., Abt. 1, Bd.1, p. 759-802.

(14) HUTCHINSON, H. B., and MILLER, N. H. J.
1911. The direct assimilation of inorganic and organic forms of nitrogen by higher plants. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 30, p. 513.

(15) JODIDI, S. L.
1909. Organic nitrogenous compounds in peat soils. Mich. Agr. Exp. Sta. Tech. Bul. 4.

(16) JODIDI, S. L.
1911. The chemical nature of the organic nitrogen in the soil. Iowa Agr. Exp. Sta. Research Bul. 1.

(17) JODIDI, S. L.
1911. The chemical nature of the organic nitrogen in the soil II. Iowa Agr. Exp. Sta. Research Bul. 3.

(18) JODIDI, S. L.
1912. Amino acids and acid amides as sources of ammonia in soils. Iowa Agr. Exp. Sta. Research Bul. 9.

(19) KELLY, W. P.
1912. The effects of calcium and magnesium carbonates on some biological transformations of nitrogen in soils. *In* Univ. Cal., Agr. Sci., v. 1, p. 39-49.

(20) KELLY, W. P.
1914. The organic nitrogen of some Hawaiian soils. I. The products of acid hydrolysis. *In* Jour. Amer. Chem. Soc., v. 36, p. 429-438.

(21) KELLY, W. P.
1915. The biochemical decomposition of nitrogenous substances in soils. Hawaii Agr. Exp. Sta. Bul. 39. States Relation Service, U. S. Dept. Agr.

(22) KOBER, P. A., and SUGIURA, K.
1913. A micro-chemical method for the determination of α- and β-amino acids and certain derivatives in proteolysis, blood and urine. *In* Jour. Amer. Chem. Soc., v. 35, p. 1546-1585.

(23) LATHROP, ELBERT C.
1912. Guanine from a heated soil. *In* Jour. Amer. Chem. Soc., v. 34, p. 1260-1263.

(24) LATHROP, ELBERT C.
1914. The nitrogen of processed fertilizers. U. S. Dept. Agr. Bul. 158.

(25) LATHROP, E. C., and BROWN, B. E.
1911. Studies in organic soil nitrogen. *In* Jour. Indus. Engin. Chem., v. 3, p. 657-660.

(26) LIPMAN, C. B., and BURGESS, P. S.
1914. Studies on ammonification in soils with pure cultures. *In* Univ. Cal., Agr. Sci. v. 1, p. 141-172.

(27) LIPMAN, J. G., BLAIR, A. W., OWEN, I. L., and MCLEAN, H. C.
1912. The availability of nitrogenous fertilizers as measured by ammonification. N. J. Agr. Exp. Sta. Bul. 246.

(28) LIPMAN, J. G., BLAIR, A. W., OWEN, I. L., and MCLEAN, H. C.
1912. Experiments on ammonia formation in the presence of carbohydrates and other non-nitrogenous material. N. J. Agr. Exp. Sta. Bul. 247.

(29) LIPMAN, J. G., BLAIR, A. W., OWEN, I. L., and MCLEAN, H. C.
1912. Experiments relating to the possible influence of protozoa on ammonification in the soil. N. J. Agr. Exp. Sta. Bul. 248.

(30) LIPMAN, J. G., BLAIR, A. W., OWEN, I. L., and MCLEAN, H. C.
1912. Conditions affecting the availability of nitrogen compounds in vegetation experiments. N. J. Agr. Exp. Sta. Bul. 249.

(31) LIPMAN, J. G., BLAIR, A. W., OWEN, I. L., and MCLEAN, H. C.
1912. Factors relating to the availability of nitrogenous plant-foods. N. J. Agr. Exp. Sta. Bul. 251.

(32) LIPMAN, J. G., BROWN, P. E., and OWEN, I. L.
1912. The availability of nitrogenous materials as measured by ammonification. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 31, 1911, p. 49-85.

(33) LÖHNIS, F.
1905. Untersuchung über den Verlauf der Stickstoffumsetzungen in der Ackererde. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 15, p. 430-435.

(34) LÖHNIS, F., and GREEN, H. H.
1913. Methods in soil bacteriology: VI—Ammonification in soil and solution. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 37, p. 534-562.

(35) LÖHNIS, F., and PARR, A. E.
1907. Zur Methodik der bakteriologischen Bodenunterschung, III. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 17, 1906, p. 518-528.

(36) MCLEAN, H. C., and WILSON, G. W.
1914. Ammonifying power of soil inhabiting fungi. *In* Science, v. 40, p. 140-142.

(37) MÖRNER, K. A. H.
1902. Zur Kenntniss der Bindung des Schwefels in den Proteinstoffen. *In* Ztsch. Physiol. Chem., Bd. 34, p. 207-338.

(38) MÜNTZ, A., and COUDON, H.
1893. La fermentation ammonical de la terre. *In* Compt. Rend. Acad. Sci. [Paris], t. 116, p. 395-398.

(39) OMELIANSKI, W. L., and SIEBER, N. O.
1913. Zur Frage nach der chemischen Zusammensetzung der Bakterienkörper des Azotobacter chroococcum. *In* Ztschr. Physiol. Chem., Bd. 88, p. 445-459.

(40) OSBORNE, T. B., LEAVENWORTH, C. S., and BRAUTLECHT, E. A.
1908. The different forms of nitrogen in proteins. *In* Amer. Jour. Physiol., v. 23, p. 180-200.

(41) POTTER, R. S., and SNYDER, R. S.
1915. Amino acid nitrogen of soil and the chemical groups of amino acids in the hydrolyzed soils and their humic acids. *In* Jour. Amer. Chem. Soc., v. 37, p. 2219-2227.

(42) POTTER, R. S., and SNYDER, R. S.
1915. The amino acid nitrogen of soil. *In* Jour. Indus. Engin. Chem., v. 7, p. 1049-1053.

(43) ROBINSON, C. S.
1911. Organic nitrogenous compounds in peat soils, II. Mich. Agr. Exp. Sta. Tech. Bul. 7.
(44) SACKETT, W. G.
1912. The ammonifying efficiency of certain Colorado soils. Colo. Agr. Exp. Sta. Bul. 184, p. 1-23.
(45) SCHREINER, O., and LATHROP, E. C.
1911. The distribution of organic constituents in soils. *In* Jour. Franklin Inst., v. 172, p. 145-151.
(46) SCHREINER, O., and LATHROP, E. C.
1912. The chemistry of steam heated soils. *In* Jour. Amer. Chem. Soc., v. 34, p. 1242-1259.
(47) SCHREINER, O., REED, H. S., and SKINNER, J. J.
1907. Certain organic constituents of soils in relation to soil fertility. U. S. Dept. Agr. Bur. Soils Bul. 47.
(48) SCHREINER, O., and SHOREY, E. C.
1909. The isolation of harmful organic substances from soils. U. S. Dept. Agr. Bur. Soils Bul. 53.
(49) SCHREINER, O., and SHOREY, E. C.
1910. The chemical nature of soil organic matter. U. S. Dept. Agr. Bur. Soils Bul. 74.
(50) SCHREINER, O., and SKINNER, J. J.
1912. Nitrogenous soil constituents and their bearing on soil fertility. U. S. Dept. Agr. Bur. Soils Bul. 87.
(51) SHOREY, E. C.
1905. Report of Chemist, Agr. Invest. Hawaii in 1905.
(52) SHOREY, E. C.
1906. Report of Chemist, Agr. Invest. Hawaii in 1906.
(53) SHOREY, E. C.
1912. The isolation of creatinine from soils. *In* Jour. Amer. Chem. Soc., v. 34, p. 99-107.
(54) SHOREY, E. C.
1912. Nucleic acids in soils. *In* Science, v. 35, p. 390.
(55) SHOREY, E. C.
1913. Some organic soil constituents. U. S. Dept. Agr. Bur. Soils Bul. 88.
(56) SHOREY, E. C. and WALTERS, E. H.
1914. A nitrogenous soil constituent, Tetracarbonimid. *In* Jour. Agr. Research, v. 3, p. 175-178.
(57) VAN SLYKE, D. D.
1911. The analysis of proteins by determination of the chemical groups characteristic of the different amino acids. *In* Jour. Biol. Chem., v. 10, p. 15-55.
(58) VAN SLYKE, D. D.
1915. Improvement in the method for analysis of proteins by determination of the chemical groups characteristic of the different amino acids. *In* Jour. Biol. Chem., v. 22, p. 281-285.
(59) VAN SLYKE, D. D., and BIRCHARD, F. J.
1913. The nature of the free amino groups in proteins. *In* Jour. Biol. Chem., v. 16, p. 539-547.
(60) WALTERS, E. H.
1915. The presence of proteoses and peptones in soils. *In* Jour. Indus. Engin. Chem., v. 7, p. 860.

THE OXIDATION OF SULFUR IN SOILS AS A MEANS OF INCREASING THE AVAILABILITY OF MINERAL PHOSPHATES[1]

By

JACOB G. LIPMAN, *Director*, and HARRY C. MCLEAN, *Chemist, Soil Research, New Jersey Agricultural Experiment Stations;* and H. CLAY LINT, *Research Fellow, Rutgers College*

The increase in the cost of phosphate which occurred in the fall of 1915 led the senior author of this paper to suggest the use of elementary sulfur for the purpose of rendering soluble inert phosphates. When this suggestion was made toward the end of 1915, European and American investigators had already shown that elementary sulfur is readily oxidized in the soil, and that such oxidation is largely, if not entirely, the result of biological activities. A bibliography on the subject of sulfur oxidation in soils is given elsewhere;[2] in this place it need be stated, merely, that environmental conditions play an important rôle in the activities of sulfur oxidizing microörganisms. Apart from the abundant suppy of oxygen which is obviously essential in any oxidizing reaction, moisture and the amount and quality of the organic matter are factors of direct significance. Moreover, the numbers and physiological efficiency of the organisms themselves are always of prime importance. As will be shown in the following pages, the oxidation reaction becomes gradually more intense, an indication that the sulfofying flora becomes more effective in response to a favorable environment.

It appears that there is a strong analogy between nitrification and sulfofication. In both cases the reaction is accomplished by obligate aerobes. In both instances a large amount of readily decomposable organic matter is undesirable, for the organisms prefer a medium whose organic matter had become at least partly mineralized. In both instances a relatively large amount of energy is made available in the oxidation process. In both instances an efficient oxidizing flora is developed gradually. Indeed, it is even possible that nitrification and sulfofication may be brought about by the same species of bacteria. To be sure, pure cultures of sulfofying bacteria which oxidize sulfur rather than hydrogen

[1] Received for publication June 2, 1916.

[2] "Sulfur Oxidation in Soils as Affecting the Availability of Mineral Phosphates," a thesis submitted by Harry C. McLean to the Faculty of Rutgers College for the Degree of M.Sc., May, 1916.

sulfide as the initial compound have not yet been isolated, and an intimate knowledge of them is still to be gained. Nevertheless, our present knowledge is sufficiently definite to warrant the statement that compost heaps, as well as cultivated fields, may be so treated as to provide a congenial environment for sulfofying bacteria. Under such favorable environments these organisms may be utilized for producing quantities of sulfuric acid sufficient for the transformation of large amounts of tricalcic into dicalcic or even monocalcic phosphate. There is every reason to think that the method here suggested is capable of the widest application in agricultural practice. When properly employed it should enable the farmer and the gardener to secure available phosphorus at a low cost and to provide at the same time enormous numbers of active oxidizing bacteria. The method proposed here is also an argument for the return to the practice of composting so prominent in the agriculture of fifty years ago.

Soil microbiology has made sufficient progress to justify the claim that the lowered productive capacity of much of our land is due to the neglect of the microbiological machinery of the soil. By returning to the practice of composting we shall again make possible the frequent addition to our land of very large numbers of desirable bacteria. But what is even more important, we shall learn to appreciate vividly that crops depend on microörganisms for the elaboration of available plant-food, and that a defective soil environment must depress crop yields. We shall strive, then, to make our fields approach the condition of a compost heap by the intelligent use of lime, green manures and chemical manures.

The present paper is in the nature of a preliminary communication. Other communications on sulfofication and sulfofying bacteria will be forthcoming. Meanwhile, proof is submitted in the following pages that large quantities of citrate-soluble phosphates may be produced in soils or soil mixtures to which there has been added finely divided sulfur and finely ground phosphate rock or other finely divided tricalcic phosphate. The commercial possibilities of the proposed method hardly need elaboration.

The sulfofication experiments recorded here were carried out in three media, one of them pure sea sand, one a tenacious red silt loam, and the third a medium loam commonly designated as Sassafras loam. The red silt loam contained 0.297 per cent P_2O_5; 10.14 per cent Fe_2O_3 and Al_2O_3, and 3.04 per cent organic carbon. The Sassafras loam contained 0.1950 per cent P_2O_5; 5.39 per cent Fe_2O_3 and Al_2O_3, and 1.07 per cent of organic carbon. It will be noted that the red silt loam was quite rich in organic carbon and phosphorus. This was due largely to composted manure which had been mixed with the soil preparatory to its use in the greenhouse for the growing of roses and carnations.

In arranging for the sulfofication experiments, quantities of the three soil media described above were thoroughly air-dried and passed through a sieve containing ten meshes to the lineal inch. The ground phosphate rock or "floats" employed in this experiment was derived from brown Tennessee rock containing 30 per cent of P_2O_5 and fine enough to pass to the extent of 95 per cent through a sieve which had 10,000 meshes to the square inch.

As shown in the accompanying table, 100-gm. quantities of soil in glass tumblers were used as the medium for sulfofication. The mixtures of rock phosphate and sulfur were made in accordance with the plan given and water was added up to 50 per cent of the water-holding capacity of the soil. In order to insure proper inoculation and to furnish at least some mineral food to the bacteria in the sand cultures, there was added to the mixture in each tumbler 10 c.c. of a soil infusion prepared by shaking for 10 minutes 100 gm. of fertile soil with 200 c.c. of a synthetic culture medium which contained no phosphorus. The tumblers and contents were then weighed and the weights recorded. From time to time the tumblers were reweighed and the moisture that had been lost by evaporation was restored. During the progress of the experiment the tumblers, covered with Petri dish covers, were kept in a dark closet at a temperature of 22° C. to 24° C.

Determinations of acidity, and of citrate-soluble and water-soluble phosphoric acid were made at the end of each week during the first eight weeks of the experiment. After that the determinations were made at intervals of two weeks. The results secured are recorded in Table I.

TABLE I

THE INFLUENCE OF SULFOFICATION ON THE ACCUMULATION OF AVAILABLE PHOSPHORIC ACID IN 15 WEEKS

Tumbler No.	Soil Medium and Treatment	P_2O_5 soluble in Ammonium Citrate		P_2O_5 soluble in Water	
		Average mg.	Inc. over ck. mg.	Average mg.	Inc. over ck. mg.
1, 2	Sand, 5 gm. Sulfur	33.80	.....	.....	.....
3, 4	Sand, 15 gm. Floats	116.47	.....	15.10	.....
5, 6	Sand, 5 gm. Sulfur, 15 gm. Floats	400.68	284.21	185.23	170.13
7, 8	Red Silt Loam, 5 gm. Sulfur	160.10	.....	.....	.....
9, 10	Red Silt Loam, 15 gm. Floats	138.77	.....	30.10	.....
11, 12	Red Silt Loam, 5 gm. Sulfur, 15 gm. Floats	1982.40	1843.63	999.56	969.46
13, 14	Sassafras Loam, 5 gm. Sulfur	101.40	.....	.....	.....
15, 16	Sassafras Loam, 15 gm. Floats	168.50	.....	18.40	.....
17, 18	Sassafras Loam, 5 gm. Sulfur, 15 gm. Floats	867.30	698.80	178.11	159.71

It is clearly shown by the amount of citrate-soluble as well as water-soluble phosphoric acid found at the end of 15 weeks that there was a very pronounced oxidation of the sulfur added and that the resulting sulfuric acid had reacted with the tricalcic phosphate. It is also apparent that the character of the soil employed, particularly as regards its mechanical and chemical composition, played an important part in stimulating or retarding the activities of the sulfofying bacteria. For instance, the sand contained at the end of 15 weeks 400.68 mg. of citrate-soluble phosphoric acid, and 185.23 mg. of water-soluble phosphoric acid where 5 gm. of sulfur and 15 gm. of floats were employed. Under the same conditions there were found in the red silt loam 1982.40 mg. of citrate-soluble and 999.56 mg. of water-soluble phosphoric acid. In the Sassafras loam the oxidation processes were not as intense as in the red silt loam, but much more intense than in the sand. However, in order to appreciate fully the influence of the soil medium on the rate of sulfur oxidation, one must compare the data secured at the end of each week within the first eight weeks, and at the end of each subsequent two weeks for seven weeks more. The rate of accumulation of sulfuric acid as affected by the aeration of the medium and the reaction of the acid formed with the basic material present will then be more clearly understood. A clearer understanding will also be had of the fact that the intensity of sulfur oxidation gradually gathers momentum under conditions favorable for the development of a strong sulfofying flora. Such favorable conditions of necessity encourage a more rapid multiplication of the microörganisms and probably also the establishment of the most effective strains or species of sulfofiers. It is possible also that associative action between sulfofying and non-sulfofying microörganisms plays a part in determining the type and degree of sulfur oxidation. The data presented in Table II serve to show the influence of each soil on the accumulation of acid.

In the sand the accumulation of acid in 15 weeks was equivalent to 100 c.c. of N/50 potassium hydrate. This occurred in the soil portions to which sulfur had been added. On the other hand, the amounts of acid found in the soil portions to which no sulfur had been added were quite small. It is interesting to note, at the same time, that, in the soil portions which had received additions of both floats and sulfur, the accumulation of acid reached, at the end of the tenth week, an equivalent of 338 c.c. of N/50 potassium hydrate. It seems, therefore, that, the presence of the tricalcic phosphate did not decrease the accumulation of acid up to a certain point. Possibly the presence of available phosphate stimulated the activities of the sulfur oxidizing bacteria so that actually more sulfuric acid was produced than in the soil portions to which no phosphate had been added. It should be noted, also, that the maximum accumula-

TABLE II

THE ACCUMULATION OF ACID AND AVAILABLE PHOSPHATES IN THREE SOIL MEDIA IN A PERIOD OF 15 WEEKS

SAND

Time	Additions: None		5 gm. Sulfur		15 gm. Floats		5 gm. Sulfur 15 gm. Floats	
	Acidity c.c. N/50 KOH	P_2O_5 mg.	Acidity c.c. N/50 KOH	P_2O_5 mg.	Acidity c.c. N/50 KOH	P_2O_5 mg.	Acidity c.c. N/50 KOH	P_2O_5 mg.
Beginning	6.00	34.58	7.25	35.06	5.75	136.63	7.50	139.04
End of 1st week	6.00	33.85	12.00	35.06	6.00	169.26	9.50	171.68
End of 2nd week	....	....	....	....	7.25	139.00	21.50	180.38
End of 3rd week	8.00	32.64	34.50	33.37	7.50	177.72	46.50	160.80
End of 4th week	....	....	....	....	8.25	187.39	143.00	234.55
End of 5th week	6.50	27.81	41.50	28.23	6.50	168.05	174.50	224.62
End of 6th week	....	....	....	....	7.75	122.10	177.00	272.02
End of 7th week	7.50	25.39	58.00	24.18	9.50	136.62	215.00	258.73
End of 8th week	....	....	....	....	7.00	136.70	208.00	262.87
End of 10th week ...	7.50	31.43	96.00	34.52	7.50	136.62	338.00	337.31
End of 12th week ...	....	....	96.00	....	7.00	138.20	336.00	390.62
End of 15th week ...	....	....	100.00	33.80	....	116.47	328.50	400.68
Increase	....	....	92.75	....	....	....	321.00	261.64

RED SILT LOAM

Beginning	2.50	111.95	5.00	105.18	4.25	143.39	5.50	142.66
End of 1st week	2.00	111.71	8.75	96.72	4.25	166.84	11.50	166.44
End of 2nd week	....	....	....	....	3.00	176.51	18.75	203.11
End of 3rd week	4.75	122.11	42.75	120.90	6.25	191.02	89.00	272.75
End of 4th week	....	....	....	....	6.00	206.74	224.00	227.29
End of 5th week	2.75	79.79	137.00	100.34	2.25	166.13	209.00	230.92
End of 6th week	....	....	....	....	6.00	148.21	281.00	258.73
End of 7th week	5.25	83.42	310.60	103.49	6.00	157.17	324.00	253.89
End of 8th week	....	....	....	....	5.50	160.79	352.00	320.38
End of 10th week ...	6.50	105.18	4404.00	154.75	5.50	153.54	646.00	340.94
End of 12th week ...	....	....	4560.00	....	....	....	596.00	396.88
End of 15th week ...	....	....	4700.00	160.10	....	138.77	710.00	1982.40
Increase	....	....	4695.00	....	....	....	704.50	1839.74

SASSAFRAS LOAM

Beginning	6.00	79.79	6.50	83.42	5.55	155.96	7.50	154.27
End of 1st week	9.50	77.37	11.25	94.30	10.00	162.01	10.00	164.91
End of 2nd week	....	....	....	....	10.00	191.01	11.50	222.46
End of 3rd week	6.00	120.90	33.50	125.74	6.25	171.68	107.50	209.16
End of 4th week	....	....	....	....	7.00	180.14	360.50	269.61
End of 5th week	7.50	99.14	454.50	103.97	5.25	165.63	353.00	342.15
End of 6th week	....	....	....	....	6.50	155.75	421.00	406.22
End of 7th week	7.00	71.33	597.00	89.47	10.50	158.38	485.00	339.73
End of 8th week	....	....	....	....	8.50	175.67	478.00	508.98
End of 10th week ...	7.00	96.72	1240.00	100.35	8.50	158.86	660.00	518.66
End of 12th week ...	....	....	1300.00	....	....	....	637.00	655.20
End of 15th week ...	....	....	1440.00	101.40	....	168.50	570.25	867.30
Increase	....	....	1433.50	....	....	....	562.75	713.03

tion of acid was found at the end of the tenth week. After that, the amount of acid found in the soil portions similarly treated was practically constant.

In the case of the red silt loam there was an increase of acid in the soil portions to which sulfur alone was added up to the end of the fifteenth week. At that time the total amount of acid found was equivalent to 4700 c.c. of N/50 potassium hydrate. The most striking increase was made between the seventh and the tenth week, when the acid increased from an equivalent of 310.60 c.c. of N/50 potassium hydrate to an equivalent of 4404 c.c. of N/50 potassium hydrate. Beyond that, the increase was but slight. When both sulfur and floats were added to the soil portions, there was a greater amount of acid accumulated in the first seven weeks of the experiment than there was in the corresponding soil portions to which sulfur alone was added. On the other hand, we find that, at the end of the tenth week, the total amount of acid found in the soil portions to which both sulfur and floats were added was equivalent to 646 c.c. of N/50 potassium hydrate as against 4404 c.c. in the soil portions to which sulfur alone was added. Beyond that point there was comparatively little change in the acidity of the soil portions that had received additions of both sulfur and floats.

In the case of Sassafras loam soil, there was also a very marked accumulation of acid in the soil portions which had addditions of sulfur only. The increase was gradual up to the end of the fifteenth week. At that time it was equivalent to 1440 c.c. of N/50 potassium hydrate. Where both floats and sulfur were used, the increase in acidity was more marked at first than it was in the corresponding portions to which sulfur alone was added. Later on, however, the accumulation in the sulfur portions became greater than in the sulfur-floats soil portions. Thus, at the end of the tenth week, the sulfur-floats portions contained an equivalent of 660 c.c. N/50 potassium hydrate as against 1240 c.c of N/50 potassium hydrate. After the end of the tenth week there was, if anything, a slight decline in the amount of acid found in the sulfur-floats portions.

The amounts of available phosphoric acid found in the three types of soil media indicate that the sulfuric acid produced in the oxidation of the sulfur had reacted with the tricalcic phosphate. Reference has already been made to the amounts of available phosphoric acid found at the end of 15 weeks in each of the three soil media employed. It need only be added here that, in the case of the sand, the total amount of available phosphoric acid at the end of the tenth week was equivalent to 337.31 mg. Beyond that the increase was relatively small. In the case of the red silt loam, the amount of available phosphoric acid at the end of the tenth week was equivalent to 340.94 mg. The striking increase came from the twelfth to the fifteenth week, when the total amount of

phosphoric acid found was equivalent to 1982.40 mg. In the Sassafras loam the increase in the amount of available phosphoric acid was more gradual. At the end of the tenth week an equivalent of 518.66 mg. of phosphoric acid was found in each soil portion which had received additions of both floats and sulfur, while, at the end of the fifteenth week, the corresponding amount found was 867.30 mg. It would seem, therefore, that the oxidation of sulfur in soils of different types is intimately dependent upon the number and physiological efficiency of sulfofying bacteria. These in their turn are readily affected by the mechanical and chemical composition of the soil medium employed.

Summary

1. Elementary sulfur is readily oxidized in soils containing sulfofying bacteria and offering favorable conditions for the development of these organisms.

2. The oxidation of sulfur in soils may lead to the accumulation of large quantities of sulfuric acid.

3. The sulfuric acid formed in the oxidation of sulfur by bacteria readily reacts with basic substances.

4. Tricalcic phosphate, when added to soils or soil mixtures in which sulfofication is active, may react with the sulfuric acid formed, and may then furnish available phosphoric acid to crops.

5. The facts recorded above justify the claim that compost heaps in which sulfofication is active may be utilized for the production of available phosphoric acid out of insoluble phosphates.

THE EFFECT OF SOIL REACTION ON AMMONIFICATION BY CERTAIN SOIL FUNGI[1]

By

NICHOLAS KOPELOFF, *Research Fellow, Rutgers College*

INTRODUCTION

The development of the science of soil biology has been marked, from time to time, by the direction of attention towards different groups of microörganisms, the bacteria, protozoa and fungi. Despite the more or less tacit understanding that fungi have various functions to perform in the soil, they have received comparatively little consideration at the hands of the soil biologist. Without assuming any exaggerated importance in their behalf, there is good reason to believe that they are a significant factor in the decomposition which takes place in soils. It appears that fungi are particularly active in the early stages of the decomposition of both nitrogenous and non-nitrogenous organic matter. It has previously been pointed out (11) that many soil fungi have a high ammonifying efficiency. This fact may be interpreted as indicating that this group of microörganisms has a bearing on the problems of soil fertility.

Obviously enough, the environmental conditions are of paramount importance in influencing their physiological activities, and principal among these is reaction. A general consideration of the occurrence, distribution and activities of soil fungi is not sufficiently pertinent to the subject at hand to necessitate any further review than has already appeared (6, 23, 24).

However, it is of interest to note that Fischer (9), Oudenmans and Koning (16), Ramann (18), and Faelli (7), have reported the occurrence of fungi in acid soils having a high organic content. Hall, Miller and Gimingham (10) found that the decline in fertility of plots which had become acid through continued use of ammonium sulfate could be attributed to the repression of the normal bacterial activities of the soil and the encouragement of molds. Marchal (15) also states that soils having a weakly alkaline or neutral reaction have relatively few fungi. Fellers (8) found that heavy soils gave the highest fungi counts on certain agar media having a reaction of 1 to 1½ per cent acidity (HCl). Alkaline media were injurious to the growth of these organisms. *Asper-*

[1] Part II of thesis submitted in partial fulfillment of the requirements for the degree of M.Sc.

Received for publication April 28, 1916.

gillus niger, Cladosporium epiphyllum, Penicillium viridicatum and *Trichodermae* all attained their maximum growth on acid media, while alkalinity proved distinctly unfavorable. This investigator notes that all the fungi studied appeared to have a wide range of reaction tolerance.

In general it is an accepted fact that neutral to acid soils are most congenial to the development of soil fungi, while an alkaline condition is for the most part, unfavorable. There is then something of a balance maintained between the number of fungi and bacteria as determined by the reaction of the soil. Or, in other words, where acidity prevails, there is a tendency for the bacteria to diminish and for the fungi to increase accordingly, while with soils having an alkaline reaction, there would be a relatively greater number of bacteria than fungi. Since it has been shown by the investigators previously mentioned that soil fungi have marked ability in producing ammonia from organic nitrogenous materials, it may reasonably be inferred that under conditions which are unfavorable to the development of great numbers of bacteria, the soil fungi would assume a considerable degree of importance in maintaining the fertility of soils. Thus in acid soils the production of ammonia by soil fungi would compensate for the reduced bacterial activities.

The purpose of the following experimentation was to determine the effect of varying soil reaction upon the ammonification of organic nitrogenous materials by certain soil fungi.

Methods

Two-hundred-c.c Erlenmeyer flasks containing 100-gm. portions of two soils, designated as Norfolk sandy loam, and Penn clay loam, respectively, were employed throughout this work. Dried blood and cottonseed meal in quantities equivalent to 155 mg. N. were used as sources of organic nitrogenous material to be ammonified. The soil after being treated was thoroughly mixed by shaking in a receptacle adapted to that purpose (12). With Norfolk sandy loam the series of flasks containing dried blood received 16.7 c.c. of water, while the series containing cottonseed meal received 20.5 c.c. With Penn clay loam the series containing dried blood received 29.7 c.c. of water, while the series containing cottonseed meal received 33.5 c.c. Thus in all cases the soil was kept at a moisture content very slightly above the optimum. Proper deduction was made in all cases for inoculum or any other liquid added. After the reaction of the soil had been altered according to the plan to be discussed presently, the flasks containing the soil were placed in the autoclave for 15 minutes at 15 pounds pressure. (This process was responsible for a loss of approximately 2 c.c. of moisture per flask.) Upon cooling, 1 c.c. of spore-suspension of the desired organism, prepared and counted according to the method described in detail in Part I (11), was inoculated into the soil, and the flasks incubated at 20° to 22° C. for 7 days, except

in the case of the *Penicillium*, where a 12-day period was found to be necessary. At the end of this time the contents of the flasks were examined for bacterial contamination by plating a small portion of the soil on Lipman and Brown's synthetic agar (13). (This practice was later discontinued, since the variation between duplicate determinations furnished an adequate criterion, in the few cases where contamination occurred.) The soil was then transferred to copper flasks, the ammonia distilled according to the magnesium oxide method and titrated with N/10 acid and alkali.

The fungi used were isolated in pure pedigree culture from soil on the College Farm and were tentatively identified as *Rhizopus nigricans*, Ehrenberg; *Zygorrhyncus Vuilleminii*, Namyslowski; and *Penicillium* sp. 10. These three organisms have been found to be present in soils by most investigators who have isolated soil fungi (24). (This particular *Penicillium* is to be considered as representative of a group of green soil *Penicillia*.) The organisms under consideration are sufficiently different in their morphology and physiological activities to offer some basis for generalization. The following work, however, must be considered under the limitations necessitated by studies with pure cultures, namely: it is questionable whether these organisms would act in the same manner when associated with other fungi, or even bacteria. Secondly, the soil as a culture medium, in the process of sterilization, undergoes certain changes which might be responsible for peculiarities not permitting of an absolute correlation with actual field conditions (5). The investigation under discussion may best be divided into two sections. The first part deals with the effect of soil reaction on ammonification by fungi, where the reaction of the soil has been altered by the addition of normal solutions of hydrochloric acid or sodium hydroxide. In the second part, the reaction of the soil has been altered by the addition of calcium carbonate (c. p.) or a normal solution of sulfuric acid.

I

The Effect of Soil Reaction on Ammonification by Certain Soil Fungi, When the Reaction Has Been Altered by Additions of Normal Solutions of HCl or NaOH

Since the problem at hand is chemical in its nature, it seemed advisable to alter the reaction of the soil by materials which would not function as food for the fungi concerned. Therefore HCl and NaOH were desirable for this purpose, as it is an established fact that none of the ions present in the above chemicals is an essential nutrient for fungi. Furthermore, in order to ensure against the possibility of either the Na or the Cl ions causing undue stimulation or depression, a solution of NaCl (3 N) was added to all the flasks in an amount approximately equivalent to the highest Na or Cl application.

Clark (4) states that the Cl ion is relatively harmless to molds and that OH is more toxic than ionic H to *Penicillium glaucum*. So far as the writer has been able to determine there is no record other than the work of McLean and Wilson (14) of any systematic experiments dealing with the alteration of reaction in the soil as affecting either the growth or the physiological activities of soil fungi.

Thom (22) reports studies with *Penicillia* where normal NaOH and normal lactic acid were added to tubes containing 10 c.c. of medium neutral to phenolphthalein. It was found that the range of tolerance in the species studied was from 2 c.c. of NaOH per 10 c.c. of medium, to 5 c.c. of acid per 10 c.c. of medium. Within this extreme range most species are more closely restricted. Very few species grow to any degree in plates alkaline to phenolphthalein. Of common green species but few fruited freely in alkali as strong as N/10. Nearly all grew best between the neutral point and an acidity approximately equal to N/10. He further suggests that this inhibiting effect of acidity varies with the species and the kind of acid used. Stevens (21) finds that the *Penicillium* spores which he studied grew in N/50 HCl and N/50 H_2SO_4, also in 2 N NaCl, solutions, but failed to grow in N/40 NaOH. Traaen (23) found that with most of the fungi he studied, N/150 to N/50 acid inhibited growth. Trichoderma appeared to be more resistant. He states further that HCl was less toxic than HNO_3. Beck (1) found that fungi which grew sparingly in N/10 HCl did not affect the titre of the acid.

It could hardly be expected that small amounts of acid or alkali would prove as toxic in soil containing a considerable supply of moisture, as in solutions such as noted by these investigators. In this experiment the Norfolk sandy loam used (for *Rhizopus* and *Zygorrhyncus*) had a lime requirement of 400 pounds of CaO per acre, on the basis of 3,000,000 pounds of soil per surface 6 2/3 inches, while that of the Penn clay loam was 1,700 pounds of CaO per acre on the basis of 2,700,000 pounds of soil per surface 6 2/3 inches. In order to approximate, as closely as possible, actual field conditions, the treatment in both the dried blood and cottonseed meal series consisted of increasing the acidity of the soil from the neutral point, in amounts equivalent to 1,000 pounds of CaO per acre, up to 4,000 pounds. Similarly, the soil was made basic by the addition of CaO up to 4,000 pounds per acre. (In the remainder of this discussion, such conditions will be referred to as "alkaline.") Most normal soils fall quite readily within these limits.

Sufficient normal HCl or NaOH was added to bring about the desired reaction. Thus in the column marked "Treatment" in Table I, "Acid $\Rightarrow$ 400 lbs. CaO" represents the original reaction of the sandy soil, while to obtain an acidity of 1,000 pounds of CaO per acre, it is evident that an addition of acid equivalent to 600 pounds CaO per acre was required, or 0.72 c.c. HCl (N/1). For an acidity of 2,000 pounds per acre, 1.92 c.c.

HCl was required, i. e., 1.2 c.c. of normal acid or alkali is equivalent to 1,000 pounds CaO per acre. Thus to obtain an alkalinity of 1,000 pounds CaO per acre, it was necessary to add 1.68 c.c. NaOH (N/1). Five-tenths of a cubic centimeter of NaCl (3 N) solution was added to all flasks.

The release of ammonia from the soil as a result of the highest application of acid or alkali did not exceed 1 to 2 mg. N, and therefore this amount was not deducted from the checks. In the following discussion, continued reference will be made to "1,000 pounds acid," "1,000 pounds alkaline," etc. It is to be assumed that "pounds of CaO per acre" is understood. The results recorded in the subsequent tables represent the two more closely agreeing determinations of an experiment carried out in triplicate.

TABLE I

THE EFFECT OF REACTION ON RHIZOPUS NIGRICANS IN NORFOLK SANDY LOAM (HCl—NaOH)

No.	Organic Matter	Treatment	NH_3 accumulated in			Increase over check
			Mg. N.	Mg. N.	Av. Mg. N.	Mg. N.
	155 mg. N.					
1-2	Dried Bl'd	Check	3.39	3.55	3.47	.....
3-4	"	Soil L. R. = 400 lbs. CaO	30.70	30.70	30.70	27.23
5-6	"	Acid = 1000 lbs. CaO....	42.46	39.40	40.93	37.46
7-8	"	Acid = 2000 lbs. CaO....	42.54	43.88	43.21	39.74
9-10	"	Acid = 3000 lbs. CaO....	19.45	21.18	20.31	16.84
11-12	"	Acid = 4000 lbs. CaO....	13.49	8.44	10.96	7.49
13-14	"	Neutral	31.95	31.24	31.58	28.11
15-16	"	Alk. = 1000 lbs. CaO.....	26.08	24.36	25.22	21.75
17-18	"	Alk. = 2000 lbs. CaO.....	19.88	19.88	19.88	16.41
19-20	"	Alk. = 3000 lbs. CaO.....	10.70	13.91	12.31	8.84
21-22	"	Alk. = 4000 lbs. CaO.....	13.49	14.73	14.11	10.64
	155 mg. N. Cottonseed					
23-24	Meal	Check	3.69	3.69	3.69	.....
25-26	"	Soil L. R. = 400 lbs. CaO	42.97	43.38	43.17	39.48
27-28	"	Acid = 1000 lbs. CaO....	39.52	41.05	40.28	36.59
29-30	"	Acid = 2000 lbs. CaO....	33.18	25.70	29.44	25.75
31-32	"	Acid = 3000 lbs. CaO....	27.06	26.15	26.60	22.91
33-34	"	Acid = 4000 lbs. CaO....	26.10	23.83	24.96	21.27
35-36	"	Neutral	36.83	39.21	38.02	34.33
37-38	"	Alk. = 1000 lbs. CaO.....	29.96	30.95	30.45	26.76
39-40	"	Alk. = 2000 lbs. CaO.....	25.53	27.83	26.65	22.96
41-42	"	Alk. = 3000 lbs. CaO.....	24.08	24.85	24.46	20.77
43-44	"	Alk. = 4000 lbs. CaO.....	6.82	7.82	7.32	3.63

An examination of Table I, which gives the effect of soil reaction on ammonification by *Rhizopus nigricans* in Norfolk sandy loam reveals the fact that in the dried blood series there is a sharp increase in ammonia accumulated where the reaction of the soil is 1,000 pounds acid compared with an acidity of but 400 pounds. There is, further, a slight increase in ammonia as the acidity is increased to 2,000 pounds, and thereafter a striking decrease is noted as the acidity is raised to 3,000 and 4,000 pounds, respectively.

In Plate I it will be seen that the mycelial growth is directly correlated with the curve of ammonia accumulation. Where the soil is made alkaline there is a gradual decline in ammonia from the neutral point with each successive application equivalent to 1,000 pounds CaO per acre until 3,000 pounds is reached. Four thousand pounds gives practically the same yield as 3,000.

Plate II, illustrating the effect of alkalinity on mycelial growth, correlates with and may be considered as the graphic representation of the curve of ammonia accumulation in this series. In the series where cottonseed meal was used as a source of organic matter, it will be seen that so far as ammonia accumulation is concerned there is a gradual decrease with successive 1,000-pound applications of acidity. Plate III, however,

TABLE II

THE EFFECT OF REACTION ON RHIZOPUS NIGRICANS IN PENN CLAY LOAM (HCl—NaOH)

No.	Organic Matter	Treatment	NH_3 accumulated in			Increase over check
			Mg. N.	Mg. N.	Av. Mg. N.	Mg. N.
	155 mg. N.					
89-90	Dried Bl'd	Check	4.23	4.96	4.60	.....
91-92	"	Soil L. R. ⇔ 1700 lbs. CaO	21.27	20.87	21.07	16.47
93-94	"	Acid ⇔ 400 lbs. CaO....	23.81	23.81	23.81	19.21
95-96	"	Acid ⇔ 1000 lbs. CaO....	23.23	22.79	23.01	18.41
97-98	"	Acid ⇔ 2000 lbs. CaO....	20.76	22.34	21.55	16.95
99-100	"	Acid ⇔ 3000 lbs. CaO....	17.59	15.44	16.57	11.97
101-102	"	Acid ⇔ 4000 lbs. CaO....	21.61	19.70	20.66	16.06
103-104	"	Neutral	22.05	21.61	21.83	17.23
105-106	"	Alk. ⇔ 1000 lbs. CaO.....	21.76	22.20	21.98	17.38
107-108	"	Alk. ⇔ 2000 lbs. CaO.....	17.93	17.49	17.71	13.11
109-110	"	Alk. ⇔ 3000 lbs. CaO.....	18.72	18.72	18.72	14.12
111-112	"	Alk. ⇔ 4000 lbs. CaO.....	16.76	16.76	16.76	12.16
	155 mg. N.					
	Cottonseed					
113-114	Meal	Check	5.21	4.99	5.10	.....
115-116	"	Sol L. R. ⇔ 1700 lbs. CaO	22.93	23.37	23.15	18.05
117-118	"	Acil ⇔ 400 lbs. CaO.....	25.58	25.43	25.51	20.41
119-120	"	Acid ⇔ 1000 lbs. CaO....	27.05	27.34	27.20	22.10
121-122	"	Acid ⇔ 2000 lbs. CaO....	25.87	26.17	26.02	20.92
123-124	"	Acid ⇔ 3000 lbs. CaO....	21.02	19.99	20.51	15.41
125-126	"	Acid ⇔ 4000 lbs. CaO....	17.93	19.55	18.79	13.69
127-128	"	Neutral	28.37	26.46	27.42	22.32
129-130	"	Alk. ⇔ 1000 lbs. CaO.....	26.17	25.28	25.73	20.63
131-132	"	Alk. ⇔ 2000 lbs. CaO.....	24.55	23.52	24.04	18.94
133-134	"	Alk. ⇔ 3000 lbs. CaO.....	21.76	20.87	21.32	16.22
135-136	"	Alk. ⇔ 4000 lbs. CaO.....	27.93	26.02	26.98	21.88

exhibits a gradual increase in mycelial growth from 400 to 2,000 pounds acidity, followed by a sharp decrease as a result of an application beyond this point. Thus in the present instance there is no correlation between mycelial growth and ammonia accumulation, the results regarding the latter suggesting the following interpretation. It is to be expected that the greater the mycelial growth the greater is the amount of nutrients consumed in its formation. Since ammounia accumulation must be con-

sidered as a process involving the concomitant factors of production and consumption of ammonia, it may be readily conceived how it was possible for more ammonia to have been produced with an acidity of 2,000 compared with 400 pounds, but that the ammonia thus produced was consumed by the fungus in mycelial development, in such a manner as to yield a smaller quantity of ammonia. It must be borne in mind that in biological studies of this nature, it is impossible to anticipate entirely coherent or concordant results at all times. The fact that a living organ-

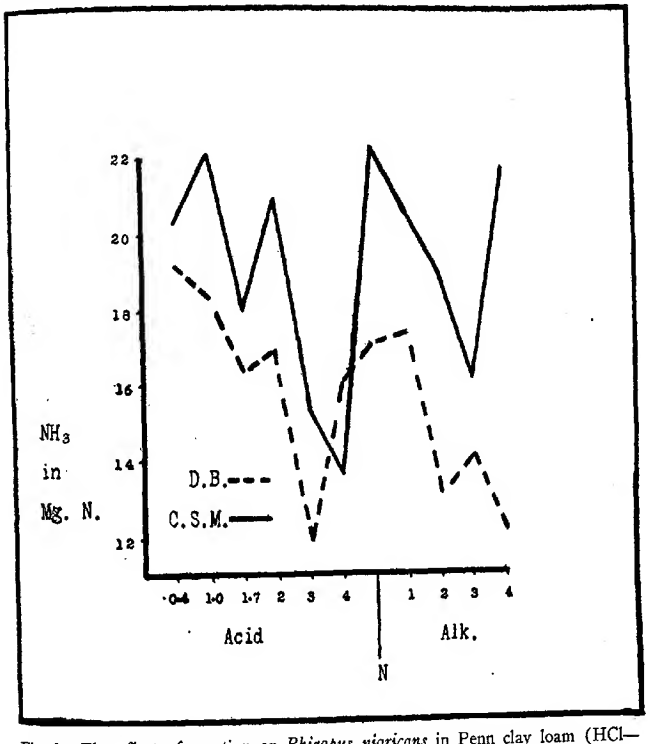


Fig. 1.—The effect of reaction on *Rhizopus nigricans* in Penn clay loam (HCl—NaOH).

ism is directly involved, which is capable of being affected by imperceptible as well as manifest variations, introduces a considerable element of uncertainty even in the chemical phases of such experimentation.

In the alkaline portion of the cottonseed meal series it will be seen that with increasing 1,000-pound applications of CaO per acre (with the exception of the initial one) there is a decrease in ammonia.

Plate IV again shows a correlation of this phenomenon with the growth of mycelium.

Regarding Table I in its entirety, then, it is evident that the reaction of the sandy soil has a profound bearing upon the ammonification of dried blood and cottonseed meal by *Rhizopus nigricans*. In accordance with the general observations heretofore mentioned, a neutral to acid reaction is most favorable to an accumulation of ammonia. However, increasing the acidity beyond 2,000 pounds causes a marked decrease in ammonia. Likewise, increasing the alkalinity causes a gradual decrease in ammonia. Consequently, so far as this organism is concerned, one of its physiological activities, ammonification, is limited by a fairly narrow range of reaction.

TABLE III

THE EFFECT OF REACTION ON ZYGORRHYNCUS VUILLEMINII IN NORFOLK SANDY LOAM

(HCl—NaOH)

No.	Organic Matter	Treatment	NH_3 accumulated in			Increase over check
			Mg. N.	Mg. N.	Av. Mg. N.	Mg. N.
	155 mg. N.					
45-46	Dried Bl'd	Check	3.39	3.55	3.47	.....
47-48	"	Soil L. R. ⇔ 400 lbs. CaO	10.65	10.65	10.65	7.18
49-50	"	Acid ⇔ 1000 lbs. CaO....	16.27	16.28	16.28	12.81
51-52	"	Acid ⇔ 2000 lbs. CaO....	24.42	25.70	25.06	21.59
53-54	"	Acid ⇔ 3000 lbs. CaO....	21.86	21.72	21.79	18.32
55-56	"	Acid ⇔ 4000 lbs. CaO....	9.75	10.22	9.98	6.51
57-58	"	Neutral	7.81	7.64	7.73	4.26
59-60	"	Alk. ⇔ 1000 lbs. CaO.....	7.45	7.52	7.49	4.02
61-62	"	Alk. ⇔ 2000 lbs. CaO.....	6.81	7.24	7.02	3.55
63-64	"	Alk. ⇔ 3000 lbs. CaO.....	7.51	7.76	7.64	4.17
65-66	"	Alk. ⇔ 4000 lbs. CaO.....	6.17	6.24	6.20	2.73
	155 mg. N.					
	Cottonseed					
67-68	Meal	Check	3.69	3.69	3.69	.....
69-70	"	Soil L. R. ⇔ 400 lbs. CaO	32.23	28.68	30.46	26.77
71-72	"	Acid ⇔ 1000 lbs. CaO....	29.18	29.39	29.29	25.60
73-74	"	Acid ⇔ 2000 lbs. CaO....	28.54	28.92	28.73	25.24
75-76	"	Acid ⇔ 3000 lbs. CaO....	22.93	22.79	22.86	19.17
77-78	"	Acid ⇔ 4000 lbs. CaO....	17.82	18.46	18.14	14.45
79-80	"	Neutral	28.81	29.11	28.96	25.27
81-82	"	Alk. ⇔ 1000 lbs. CaO.....	22.08	20.61	21.35	17.66
83-84	"	Alk. ⇔ 2000 lbs. CaO.....	13.27	14.05	13.66	9.97
85-86	"	Alk. ⇔ 3000 lbs. CaO.....	11.72	10.36	11.04	7.35
87-88	"	Alk. ⇔ 4000 lbs. CaO.....	10.36	11.28	10.82	7.13

Table II shows the effect of reaction on *Rhizopus nigricans* in Penn clay loam, a graphic representation of which appears in figure 1. In this experiment the inoculation consisted of 378,000 spores per 1 c.c. From the results of the dried blood series in Table I it will be seen that an acidity of 400 to 1,000 pounds appears to be most favorable for the accumulation of ammonia. Applications of 1,700, 2,000 and 4,000 pounds gave somewhat lower results. Again, these facts may be more readily explained after the observations on mycelial growth are recorded. Thus

the growth in flasks receiving an acidity of 3,000 pounds was greater than in those having 4,000 pounds. In all of the other instances the

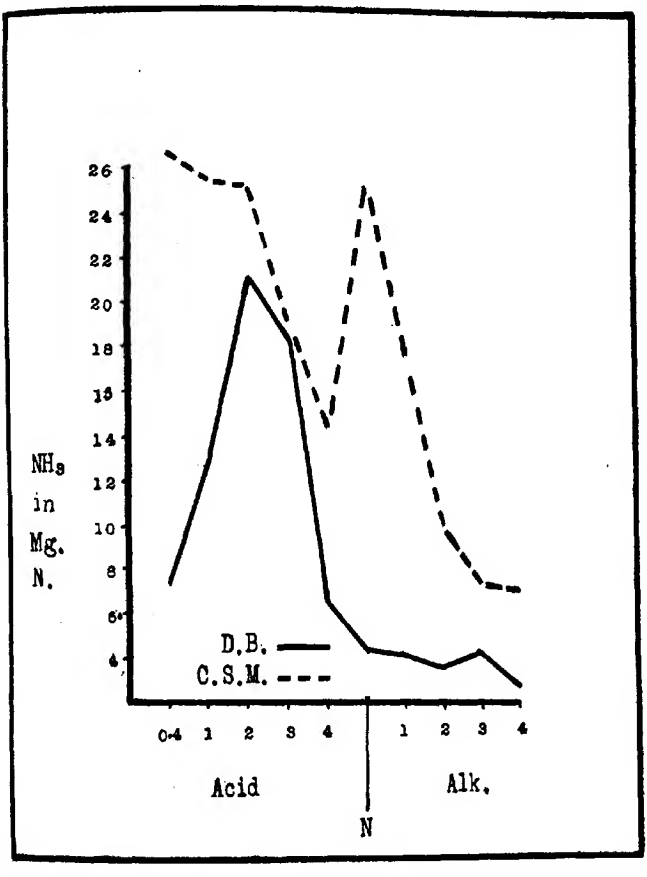


Fig. 2.—The effect of reaction on *Zygorrhyncus Vuilleminii* in Norfolk sandy loam (HCl—NaOH).

amount of mycelial growth could be correlated with ammonia accumulation. Therefore the above-mentioned exceptions would indicate that in those particular cases more ammonia may actually have been produced, but similarly more had been consumed in the development of mycelia. Thus, in effect, it might be argued that a reaction varying from neutral to 1,000 pounds acidity was the most favorable for the accumulation of

ammonia in this soil. Furthermore, there appears to be a tendency towards a decrease in ammonia as the alkalinity is increased. In the cottonseed meal series there is an increase in ammonia with 1,000 pounds acidity compared with 400 pounds. There is a gradual decrease in ammonia as the acidity is increased beyond this point. Again, it is to be noted that the mycelial growth in an acidity of 1,700 pounds was the same as that in 2,000 pounds, and therefore it may be assumed that a reaction between the neutral point and an acidity of 2,000 pounds is most favorable for the ammonification. It is evident that increasing the alkalinity causes a gradual decrease in ammonia (with but one exception).

TABLE IV

THE EFFECT OF REACTION ON ZYGORRHYNCUS VUILLEMINII IN PENN CLAY LOAM
(HCl—NaOH)

No.	Organic Matter	Treatment	NH_3 accumulated in			Increase over check
			Mg. N.	Mg. N.	Av. Mg. N.	Mg. N.
	155 mg. N.					
137-138	Dried Bl'd	Check	4.15	3.93	4.04	.....
139-140	"	Soil L. R. = 1700 lbs. CaO	10.37	9.50	9.99	5.95
141-142	"	Acid = 400 lbs. CaO....	9.65	9.50	9.58	5.54
143-144	"	Acid = 1000 lbs. CaO....	9.50	9.50	9.50	5.46
145-146	"	Acid = 2000 lbs. CaO....	12.10	10.08	11.09	7.05
147-148	"	Acid = 3000 lbs. CaO....	11.38	10.94	11.16	7.12
149-150	"	Acid = 4000 lbs. CaO....	15.12	14.54	14.83	10.79
151-152	"	Neutral	10.94	10.22	10.58	6.54
153-154	"	Alk. = 1000 lbs. CaO.....	9.36	9.94	9.65	5.61
155-156	"	Alk. = 2000 lbs. CaO.....	9.65	9.22	9.44	5.40
157-158	"	Alk. = 3000 lbs. CaO.....	9.05	8.64	8.85	4.81
159-160	"	Alk. = 4000 lbs. CaO.....	9.22	9.05	9.14	5.10
	155 mg. N. Cottonseed					
161-162	Meal	Check	3.82	5.17	4.50	.....
163-164	"	Soil L. R. = 1700 lbs. CaO	19.15	20.45	19.80	15.30
165-166	"	Acid = 400 lbs. CaO....	16.99	16.56	16.78	12.28
167-168	"	Acid = 1000 lbs. CaO....	19.01	19.01	19.01	14.51
169-170	"	Acid = 2000 lbs. CaO....	21.02	21.89	21.46	16.96
171-172	"	Acid = 3000 lbs. CaO....	20.74	19.87	20.31	15.81
173-174	"	Acid = 4000 lbs. CaO....	21.02	17.42	19.22	14.72
175-176	"	Neutral	18.43	18.29	18.36	13.86
177-178	"	Alk. = 1000 lbs. CaO.....	14.83	14.83	14.83	10.33
179-180	"	Alk. = 2000 lbs. CaO.....	14.69	14.40	14.55	10.05
181-182	"	Alk. = 3000 lbs. CaO.....	11.52	12.24	11.88	7.38
183-184	"	Alk. = 4000 lbs. CaO.....	9.65	8.64	9.15	4.65

Considering then the data presented, it will be observed that in general with *Rhizopus nigricans*, both in sandy and in clay soils, using both kinds of organic matter, there seems to be a fairly narrow range of tolerance to acidity and alkalinity so far as the maximum ammonia accumulation is concerned.

In Table III are recorded the results dealing with the effect of reaction on *Zygorrhyncus Vuilleminii* in Norfolk sandy loam, which are graphically presented in figure 2. It may be perceived that there is a striking increase in ammonia from the neutral point with an increase in

acidity up to 2,000 pounds. Any increase in acidity beyond this point is marked by a decrease in ammonia. Increasing the alkalinity causes a gradual decrease in ammonia (with but one exception). Considering the cottonseed meal series there is practically an equal amount of ammonia accumulated where the reaction ranges from neutral to 2,000 pounds acid, but an increase in acidity beyond this point is responsible for a decrease in ammonia. As previously noted, an increase in alkalinity is responsible for a gradual decrease in ammonia. Because of the fact that

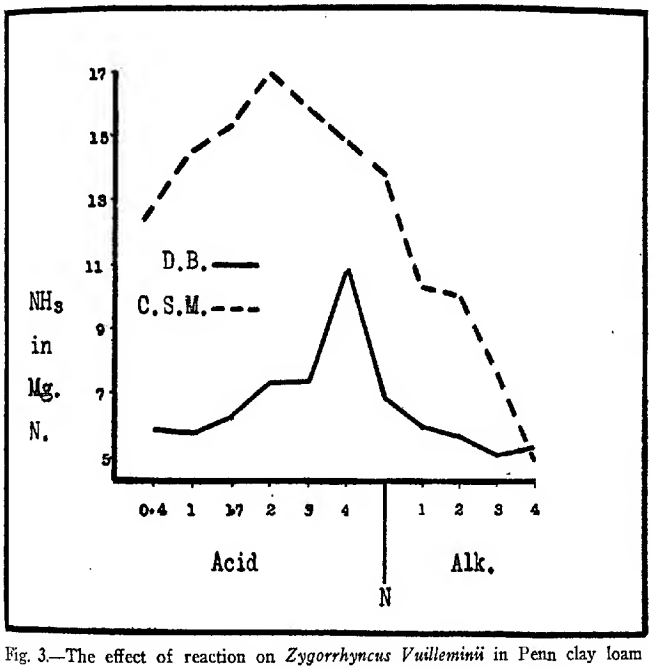


Fig. 3.—The effect of reaction on *Zygorrhyncus Vuilleminii* in Penn clay loam (HCl—NaOH).

Zygorrhynus Vuilleminii does not produce as rank a mycelial growth as *Rhizopus*, it is hardly possible to establish, with any degree of precision, a close correlation between ammonia accumulation and mycelial growth. However, the observations made substantiate the evidence that in general this correlation obtains throughout this work.

In Table IV and figure 3 are set forth the results dealing with the effect of reaction on *Zygorrhyncus Vuilleminii* in Penn clay loam. There were present 55,000 spores per 1 c.c. of inoculum, a fact which accounts for the comparatively small amounts of ammonia accumulated, especially with dried blood, which has been shown, in another connection, to be a

poor source of ammonifiable material for this organism (11). In point of fact, differences manifested by various treatments are in most instances so slight as to permit of no definite conclusions. In the cottonseed meal series, however, it is evident that there is a gradual increase in ammonia with successive increases in acidity up to 2,000 pounds, and thereafter a slight decline may be observed. Increasing the alkalinity is responsible for a gradual decrease in ammonia accumulation.

TABLE V

THE EFFECT OF REACTION ON PENICILLIUM SP. IN NORFOLK SANDY LOAM (HCl—NaOH)

No.	Organic Matter	Treatment	NH_3 accumulated in			Increase over check
			Mg. N.	Mg. N.	Av. Mg. N.	Mg. N.
185-186	155 mg. N. Dried Bl'd	Check	2.65	2.15	2.40	.....
187-188	"	Acid ⇔ 1000 lbs. CaO....	20.86	22.12	21.49	19.09
189-190	"	Acid ⇔ 2000 lbs. CaO....	29.54	30.10	29.82	27.42
191-192	"	Soil L. R. ⇔ 2300 lbs. CaO	30.94	31.50	31.22	28.82
193-194	"	Acid ⇔ 3000 lbs. CaO....	28.56	29.26	29.41	27.01
195-196	"	Acid ⇔ 4000 lbs. CaO....	12.46	12.18	12.32	9.92
197-198	"	Neutral	15.96	16.52	16.24	13.84
199-200	"	Alk. ⇔ 1000 lbs. CaO.....	8.82	9.24	9.03	6.63
201-202	"	Alk. ⇔ 2000 lbs. CaO.....	7.98	7.70	7.84	5.44
203-204	"	Alk. ⇔ 3000 lbs. CaO.....	6.02	4.76	5.39	2.99
205-206	"	Alk. ⇔ 4000 lbs. CaO.....	3.78	4.06	3.92	1.52
	155 mg. N. Cottonseed					
207-208	Meal	Check	2.71	2.89	2.80	.....
209-210	"	Acid ⇔ 1000 lbs. CaO....	19.88	20.30	20.09	17.29
211-212	"	Acid ⇔ 2000 lbs. CaO....	26.04	29.26	27.65	24.85
213-214	"	Soil L. R. ⇔ 2300 lbs. CaO	23.94	22.82	23.38	20.58
215-216	"	Acid ⇔ 3000 lbs. CaO....	23.10	22.12	22.61	19.81
217-218	"	Acid ⇔ 4000 lbs. CaO....	11.62	11.34	11.48	8.68
219-220	"	Neutral	10.64	11.76	11.20	8.40
221-222	"	Alk. ⇔ 1000 lbs. CaO.....	4.62	3.36	3.99	1.19
223-224	"	Alk. ⇔ 2000 lbs. CaO.....	3.22	3.50	3.36	0.56
225-226	"	Alk. ⇔ 3000 lbs. CaO.....	2.94	2.94	2.94	0.14
227-228	"	Alk. ⇔ 4000 lbs. CaO.....	6.58	6.16	6.37	3.57

Thus considering as a whole the data presented on the effect of reaction on ammonification by *Zygorrhyncus Vuilleminii*, in both sandy and clay soils with the two different sources of organic matter, it appears that the reaction most favorable to maximum ammonia accumulation lies between the rather narrow limits of the neutral point and an acidity of 2,000 pounds. It will be remembered that this coincides with the results obtained with *Rhizopus nigricans* under similar conditions.

In Table V and figure 4 are recorded the results dealing with the effect of reaction on ammonification by *Penicillium* sp. 10 in Norfolk sandy loam. A different sample of this type was used in this experiment with *Penicillium* from that which had been previously employed with *Rhizopus nigricans* and *Zygorrhyncus Vuilleminii*. The sole difference,

however, is to be found in the fact that the present sample had a considerably higher lime requirement, namely, 2,300 pounds CaO per acre. Also, it may be mentioned that a different sample of Penn clay loam was employed, having a lime requirement of 1,100 pounds per acre. In inoculating both sand and clay, there were present 452,000 spores per 1 c.c. of spore-suspension. The flasks were incubated for 7 days, but the ammonification was so slight as to necessitate a repetition of the experiment with a 12-day incubation, the results of which are recorded below.

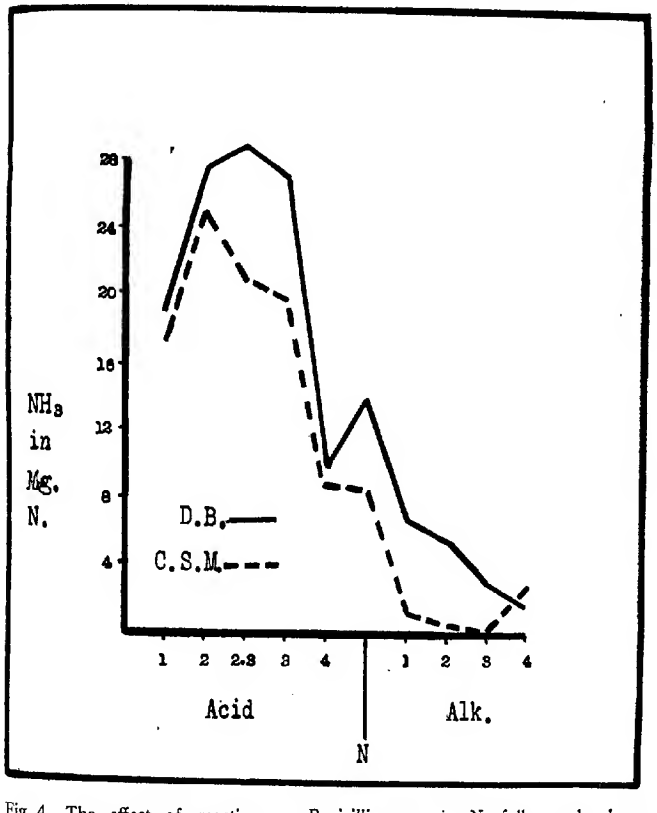


Fig. 4.—The effect of reaction on *Penicillium* sp. in Norfolk sandy loam (HCl—NaOH).

In the dried blood series there is an increase in ammonia from the neutral point to an acidity of 2,300 pounds, above which point a decline sets in. An increase in alkalinity is responsible for a pronounced decrease

in ammonia accumulated. In the cottonseed meal series there is an increase in ammonia with an increase in acidity up to 2,000 pounds, and therefore a decrease in ammonia occurs. Making the soil alkaline beyond the neutral point resulted in a slight accumulation of ammonia which may be regarded as negligible.

TABLE VI

THE EFFECT OF REACTION ON PENICILLIUM SP. IN PENN CLAY LOAM (HCl—NaOH)

No.	Organic Matter	Treatment	NH_3 accumulated in			Increase over check
			Mg. N.	Mg. N.	Av. Mg. N.	Mg. N.
	155 mg. N.					
229-230	Dried Bl'd	Check	2.91	2.69	2.80	.....
231-232	"	Acid ⇔ 1000 lbs. CaO....	25.06	26.46	25.76	22.96
233-234	"	Soil L. R. ⇔ 1100 lbs. CaO	21.70	20.86	21.28	18.48
235-236	"	Acid ⇔ 2000 lbs. CaO....	29.68	25.48	27.58	24.78
237-238	"	Acid ⇔ 2300 lbs. CaO....	32.20	29.26	30.73	27.93
239-240	"	Acid ⇔ 3000 lbs. CaO....	23.24	23.94	23.59	20.79
241-242	"	Acid ⇔ 4000 lbs. CaO....	23.94	22.68	23.31	20.51
243-244	"	Neutral	20.44	22.54	21.49	18.69
245-246	"	Alk. ⇔ 1000 lbs. CaO.....	21.98	22.26	22.12	19.32
247-248	"	Alk. ⇔ 2000 lbs. CaO.....	16.66	21.42	19.04	16.24
249-250	"	Alk. ⇔ 3000 lbs. CaO.....	18.62	18.62	18.62	15.82
251-252	"	Alk. ⇔ 4000 lbs. CaO.....	14.84	13.44	14.14	11.34
	155 mg. N. Cottonseed					
253-254	Meal	Check	3.93	3.87	3.90	.....
255-256	"	Acid ⇔ 1000 lbs. CaO....	15.26	16.94	16.10	12.20
257-258	"	Soil L. R. ⇔ 1100 lbs. CaO	19.88	17.64	18.76	14.86
259-260	"	Acid ⇔ 2000 lbs. CaO....	19.18	20.74	19.96	16.06
261-262	"	Acid ⇔ 2300 lbs. CaO....	17.08	17.36	17.22	13.32
263-264	"	Acid ⇔ 3000 lbs. CaO....	15.26	15.68	15.47	11.57
265-266	"	Acid ⇔ 4000 lbs. CaO....	12.18	11.06	11.62	7.72
267-268	"	Neutral	17.36	16.38	16.87	12.97
269-270	"	Alk. ⇔ 1000 lbs. CaO.....	13.72	13.16	13.44	9.54
271-272	"	Alk. ⇔ 2000 lbs. CaO.....	9.10	7.70	8.40	4.50
273-274	"	Alk. ⇔ 3000 lbs. CaO.....	4.90	7.28	6.09	2.19
275-276	"	Alk. ⇔ 4000 lbs. CaO.....	5.04	5.88	5.46	1.56

In Table VI and figure 5 which show the effect of reaction on ammonification by *Penicillium* sp. 10 in Penn clay loam, it will be seen that there is an increase in ammonia with an increase in acidity up to 2,300 pounds, following which a decrease ensues (with but one exception). Increasing the alkalinity causes a corresponding decrease in ammonia. In the cottonseed meal series there is an increase in acidity up to 2,000 pounds, followed by a decrease in ammonia beyond this point. Again, it is obvious that an increase in alkalinity is responsible for a marked decrease in ammonia.

Therefore in this experiment where a sandy soil of high lime requirement and a clay soil of lower lime requirement than that previously used were employed, with dried blood as the source of organic matter, the maximum ammonia accumulation occurred with a reaction varying from the neutral point to 2,300 pounds acidity. Where cottonseed meal was

used, the maximum ammonia accumulation took place at 2,000 pounds acid. It is possible that the reason for this difference is that cottonseed meal might induce a more acid condition in the soil than dried blood in this period of time. Therefore a slightly smaller addition of acidity, i. e., 2,000 pounds, to a soil receiving cottonseed meal would be as efficient as the application of an acidity of 2,300 pounds where dried blood was used, so far as maximum ammonia accumulation with this fungus was concerned. In all cases an increase in alkalinity was responsible for a decrease in ammonia accumulation.

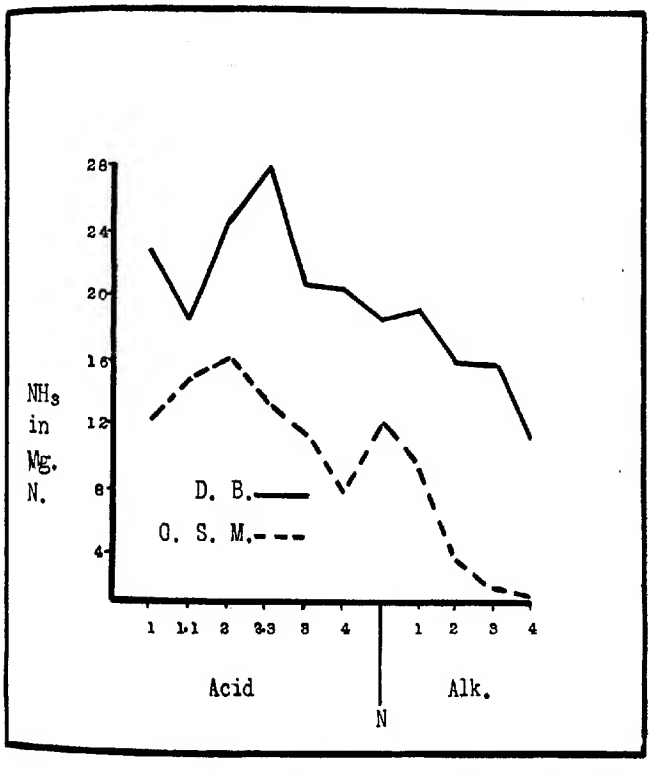


Fig. 5.—The effect of reaction on *Penicillium* sp. in Penn clay loam (HCl—NaOH).

Recapitulating the salient features brought out in the preceding discussion, the following points may be established with due regard for the limitations involved in the experiment.

1. Using normal solutions of HCl and NaOH to alter the soil reaction, it was found that the latter had a profound bearing upon the ammonification of organic nitrogenous materials by *Rhizopus nigricans, Zygorrhyncus Vuilleminii,* and *Penicillium* sp. 10, all of which were influenced in the same manner.

2. The effect of soil reaction upon the ammonification of dried blood by these fungi was practically the same as that of cottonseed meal.

3. The effect of soil reaction on the ammonification of these materials by the fungi employed was practically the same in sandy or clay loam of high or of low lime requirement.

4. The maximum ammonia accumulated by these organisms using either of the soils with either of the organic materials occurred when the reaction of the soil lay between the neutral point and an acidity equivalent to 2,000 pounds CaO per acre.

5. Increasing the acidity beyond this point, or increasing alkalinity beyond the neutral point usually was responsible for a corresponding decrease in ammonia accumulated.

6. In general, whenever such observation was possible, it was found that mycelial growth could be correlated with ammonia accumulation.

II

The Effect of Soil Reaction on Ammonification by Soil Fungi when the Reaction has been Altered by Additions of $CaCO_3$ and H_2SO_4.

Having established the range of soil reaction which was most advantageous to a maximum accumulation of ammonia by these organisms, it was deemed advisable to make the inquiry somewhat more practical in its bearing by using $CaCO_3$ instead of NaOH and substituting H_2SO_4 for the HCl previously employed. It is obvious that in actual field practice soil acidity is corrected by applications of lime. Since caustic lime has an antiseptic property, it was considered more desirable to use calcium carbonate. Sulfuric acid was chosen in preference to other acids because when used in conjunction with $CaCO_3$ it made possible the use of a neutral compound namely $CaSO_4$ which contains the most important radicals of the two materials employed. Furthermore sulfur is not used by fungi as a nutrient to as great an extent as is carbon which would be present in any organic acid that might be worthy of consideration.

Stevens (21) states that *Penicillium* spores grow in N/50 H_2SO_4 and Traaen (23) maintains that H_2SO_4 is not as toxic to fungi as HCl. Planchon (17) likewise found that H_2SO_4 was advantageous to the development of molds.

The question of whether or not calcium is an essential element of food for fungi or can function as such if replacing magnesium is at present a somewhat disputed point. Sauton (20) states that Ca cannot replace Mg as an essential element and in fact depresses fungous growth.

Robert (19) found that there was a slight increase in the weight of fungi proportional to the amount of Ca added, provided the latter were small. Winogradsky (25) states that Ca is not an essential element and cannot replace Mg. Buromsky (2) quotes the work of Molisch and others to show that Ca cannot replace Mg but increases the yield of fungous growth somewhat. According to Buromsky, Ca cannot serve as a

TABLE VII

THE EFFECT OF REACTION ON PENICILLIUM SP. IN NORFOLK SANDY LOAM (H_2SO_4—$CaCO_3$)

No.	Organic Matter	Treatment	NH_3 accumulated in			Increase over check
			Mg. N.	Mg. N.	Av. Mg. N.	Mg. N.
	155 mg. N.					
	Dried Bl'd	Check	2.53	2.27	2.40	.....
1-2	"	Acid = 1000 lbs. CaO....	22.26	23.52	22.89	20.49
3-4	"	Acid = 2000 lbs. CaO....	27.44	29.12	28.28	25.88
5-6	"	Soil L. R. = 2300 lbs. CaO	29.50	30.00	29.75	27.35
7-8	"	Acid = 3000 lbs. CaO....	26.49	29.18	27.89	25.49
9-10	"	Acid = 4000 lbs. CaO....	25.70	25.14	25.42	23.02
11-12	"	Neutral	15.26	13.44	14.35	11.95
13-14	"	Alk. = 1000 lbs. CaO.....	10.08	10.15	10.75	7.75
15-16	"	Alk. = 2000 lbs. CaO.....	8.68	7.98	8.33	5.93
17-18	"	Alk. = 3000 lbs. CaO.....	8.40	7.70	8.05	5.65
19-20	"	Alk. = 4000 lbs. CaO.....	6.72	7.56	7.14	4.74
21-22	"	Alk. = 10,000 lbs. CaO...	11.60	11.35	11.48	9.08
23-24	"	Alk. = 20,000 lbs. CaO...	9.60	9.82	9.71	7.31
25-26	"	Alk. = 30,000 lbs. CaO...	10.00	9.60	9.80	7.40
27-28	"	Alk. = 40,000 lbs. CaO...	10.00	9.85	9.93	7.53
29-30	"	Alk. = 50,000 lbs. CaO...	10.04	9.50	9.77	7.37

nutrient for fungi and depresses the yield of *Aspergillus niger*. Butkewitch (3) found that $CaCO_3$ in amounts of 2 and 10 gm. in 50 c.c. of nutrient solution containing 4 per cent peptone, 0.2 per cent sugar and 0.2 per cent NaCl depressed the production of ammonia to one-fourth of the quantity produced in the absence of $CaCO_3$. A review of the literature then indicates that Ca is not an essential nutrient for fungi and that in small amounts it may act as a stimulant, while in larger amounts it may actually depress the growth of these organisms.

In the following experiments the Norfolk sandy loam used had a lime requirement of 2,300 pounds CaO per acre on the basis of 3,000,000 pounds of soil per surface 6 2/3 inches and the Penn clay loam had a lime requirement of 1,100 pounds per acre on the basis of 2,700,000 pounds of soil per surface 6 2/3 inches. The method of procedure was identical with that previously outlined. A normal solution of H_2SO_4 and $CaCO_3$ (c. p.) were employed to alter the reaction of the soil. As before, in these experiments the same gradations obtained, except that in the alkalinity series where the applications were increased as high as 50,000 pounds CaO per acre, or practically 2 per cent, to guard against the possible influence of either the Ca or SO_4 radicals, $CaSO_4$ was applied at the outset to the flasks in quantities equivalent to the highest amounts of

those radicals used. Later this practice was considered superfluous and was discarded.

Considering the effect of reaction on *Penicillium* sp. 10 (using 132,000 spores per 1 c.c. of inoculum) in Norfolk sandy loam as shown in Table VII and figure 6 in the dried blood series, it is evident that there is a pronounced increase in ammonia accumulation as the acidity is in-

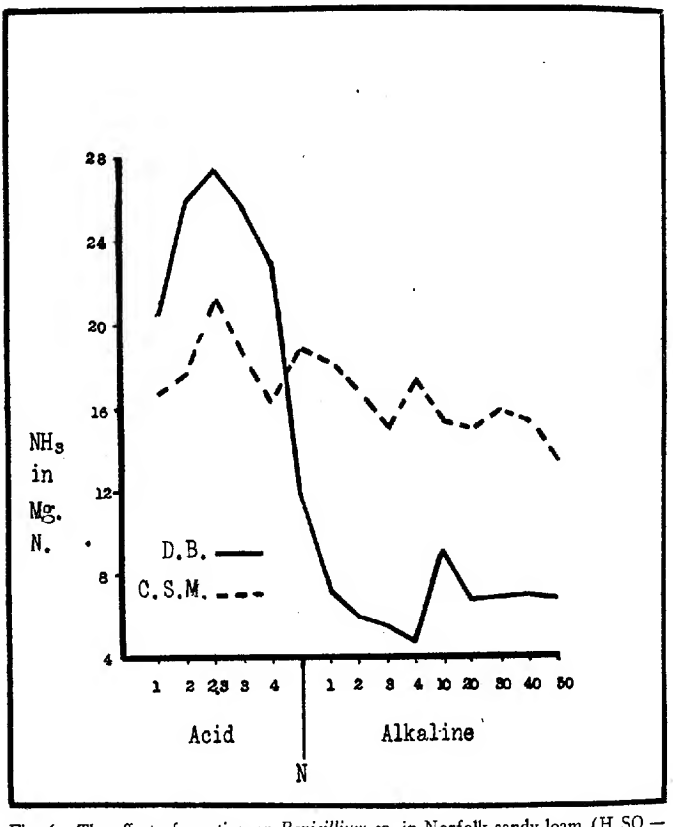


Fig. 6.—The effect of reaction on *Penicillium* sp. in Norfolk sandy loam (H_2SO_4—$CaCO_3$).

creased from the neutral point to an acidity of 2,300 pounds. Thereafter, there is a decrease in ammonia. With an increase in alkalinity beyond the neutral point, there is a decrease in ammonia, although this is not proportional to the increase in application of $CaCO_3$ above 4,000 pounds. In point of fact the addition of 10,000 to 50,000 lbs CaO per acre yielded a greater amount of ammonia than the smaller applications.

Owing to the nature of the growth of this organism in soil it does not readily permit of the correlation of mycelial growth with ammonia accumulation. Thus speculation might be advanced to the effect that the explanation of the above phenomenon depended on the greater production of ammonia, but likewise greater consumption in the process of growth.

TABLE VIII

THE EFFECT OF REACTION ON PENICILLIUM SP. IN NORFOLK SANDY LOAM (H_2SO_4—$CaCO_3$)

No.	Organic Matter	Treatment	NH_3 accumulated in			Increase over check
			Mg. N.	Mg. N.	Av. Mg. N.	Mg. N.
	155 mg. N. Cottonseed Meal	Check	2.91	2.69	2.80	.....
31-32	"	Acid ⇔ 1000 lbs. CaO....	20.44	18.62	19.53	16.73
33-34	"	Acid ⇔ 2000 lbs. CaO....	19.74	21.28	20.51	17.71
35-36	"	Soil L. R. ⇔ 2300 lbs. CaO	24.75	22.85	23.80	21.00
37-38	"	Acid ⇔ 3000 lbs. CaO....	19.43	23.10	21.27	18.47
39-40	"	Acid ⇔ 4000 lbs. CaO....	19.88	18.15	19.02	16.22
41-42	"	Neutral	21.28	21.98	21.63	18.83
43-44	"	Alk. ⇔ 1000 lbs. CaO.....	21.84	20.30	21.07	18.27
45-46	"	Alk. ⇔ 2000 lbs. CaO.....	21.56	18.90	20.23	17.43
47-48	"	Alk. ⇔ 3000 lbs. CaO.....	17.22	18.62	17.92	15.12
49-50	"	Alk. ⇔ 4000 lbs. CaO.....	20.72	20.30	20.51	17.71
51-52	"	Alk. ⇔ 10,000 lbs. CaO...	18.65	17.80	18.23	15.43
53-54	"	Alk. ⇔ 20,000 lbs. CaO...	17.89	17.85	17.87	15.07
55-56	"	Alk. ⇔ 30,000 lbs. CaO...	18.80	18.70	18.75	15.95
57-58	"	Alk. ⇔ 40,000 lbs. CaO...	18.30	17.85	18.08	15.28
59-60	"	Alk. ⇔ 50,000 lbs. CaO...	17.18	15.31	16.25	13.45

In the cottonseed meal series, the results of which are given in Table VIII and figure 6, it is again apparent that with an increase in acidity of 1,000 to 2,300 pounds there is a gradual increase in ammonia accumulation. Beyond this point there is perceptible decline. While an increase in alkalinity from 1,000 to 4,000 pounds (with one exception) causes a decrease in ammonia, at the latter point practically a constant ensues.

In Table IX and figure 7 is shown the effect of reaction on ammonification by *Penicillium* sp. 10. in Penn clay loam using dried blood as a source of organic matter. With an increase in acidity from 1,000 to 2,000 pounds there is an increase in ammonia. But a further increase to 3,000 pounds does not cause any decline such as takes place when the acidity is increased to 4,000 pounds. Increasing the alkalinity from 1,000 to 4,000 pounds causes a corresponding decrease in ammonia. Further applications have no influence since practically a constant is maintained. These results are found to be in agreement with those obtained by McLean and Wilson (14) with another species of *Penicillium* in the same soil.

In the cottonseed meal series, the results of which appear in Table X and figure 7, the maximum ammonia accumulation occurs between the neutral point and an acidity of 2,000 pounds. Above this point there is a gradual decline in ammonia accumulation. Increasing the alkalinity

causes a corresponding decrease in ammonia to 3,000 pounds, following which practically a constant is maintained.

Thus considering as a whole the data presented on the effect of reaction on ammonification by *Penicillium* in sandy and clay soils using both kinds of organic matter, it is evident that the maximum ammonia accumulation occurs with a reaction between the neutral point and an acidity of 2,300 pounds. Increasing the acidity beyond this point causes a decrease in ammonia accumulation. Again, increasing the alkalinity up to 4,000 pounds causes a decrease in ammonia. Applications beyond this point do not result in any corresponding change in ammonia, since a constant ensues which is usually about the same in quantity as that with 2,000 to 3,000 pounds. The only explanation that suggests itself is that

TABLE IX

THE EFFECT OF REACTION ON PENICILLIUM SP. IN PENN CLAY LOAM (H_2SO_4—$CaCO_3$)

No.	Organic Matter	Treatment	NH_3 accumulated in			Increase over check
			Mg. N.	Mg. N.	Av. Mg. N.	Mg. N.
	155 mg. N.					
	Dried Bld.	Check	2.73	2.87	2.80	.....
61-62	"	Acid ⇔ 1000 lbs. CaO....	18.90	20.02	19.46	16.66
63-64	"	Soil L. R. ⇔ 1100 lbs. CaO	21.50	21.15	21.33	18.53
65-66	"	Acid ⇔ 2000 lbs CaO....	23.10	22.70	22.90	20.10
67-68	"	Acid ⇔ 2300 lbs. CaO....	23.12	22.70	22.91	20.11
69-70	"	Acid ⇔ 3000 lbs. CaO....	22.85	22.98	22.92	20.12
71-72	"	Acid ⇔ 4000 lbs. CaO....	22.40	21.32	21.86	19.06
73-74	"	Neutral	18.70	20.60	19.65	16.85
75-76	"	Alk. ⇔ 1000 lbs. CaO.....	10.50	10.78	10.64	7.84
77-78	"	Alk. ⇔ 2000 lbs. CaO.....	8.26	8.54	8.40	5.60
79-80	"	Alk. ⇔ 3000 lbs. CaO.....	6.30	6.30	6.30	3.50
81-82	"	Alk. ⇔ 4000 lbs. CaO.....	5.46	5.60	5.53	2.73
83-84	"	Alk. ⇔ 10,000 lbs. CaO...	8.47	9.00	8.74	5.94
85-86	"	Alk. ⇔ 20,000 lbs. CaO...	7.70	7.77	7.74	4.94
87-88	"	Alk. ⇔ 30,000 lbs. CaO...	8.28	8.10	8.19	5.39
89-90	"	Alk. ⇔ 40,000 lbs. CaO...	7.78	7.84	7.81	5.01
91-92	"	Alk. ⇔ 50,000 lbs. CaO...	7.65	7.75	7.70	4.90

possibly the addition of such large quantities of $CaCO_3$, may improve the texture of the soil to such an extent as to make the increased oxygen supply an advantageous factor in ammonia accumulation.

Considering the effect of reaction on ammonification by *Zygorrhyncus Vuilleminii* (using 32,000 spores per 1 c.c. of inoculum) in Norfolk sandy loam where dried blood was used, as shown in Table XI and figure 8, it is evident that with an increased acidity from the neutral point to 2,000 pounds, there is a corresponding increase in ammonia. Above the latter point, a further increase in acidity causes a decrease in ammonia. An increase in alkalinity beyond the neutral point allows of a negligible amount of ammonia accumulation.

In the cottonseed meal series as shown in Table XII and figure 8, an increase in acidity from the neutral point to 2,000 pounds causes a cor-

responding increase in ammonia accumulation. Beyond the latter point there is a decline in ammonia. In all probability the fact that more ammonia was accumulated in the presence of an acidity of 4,000 than was the case with 3,000 pounds is due to a greater consumption of ammonia in the latter case. With increasing alkalinity there appears to be a tendency towards a diminution of ammonia, but the variations permit of no definite conclusion.

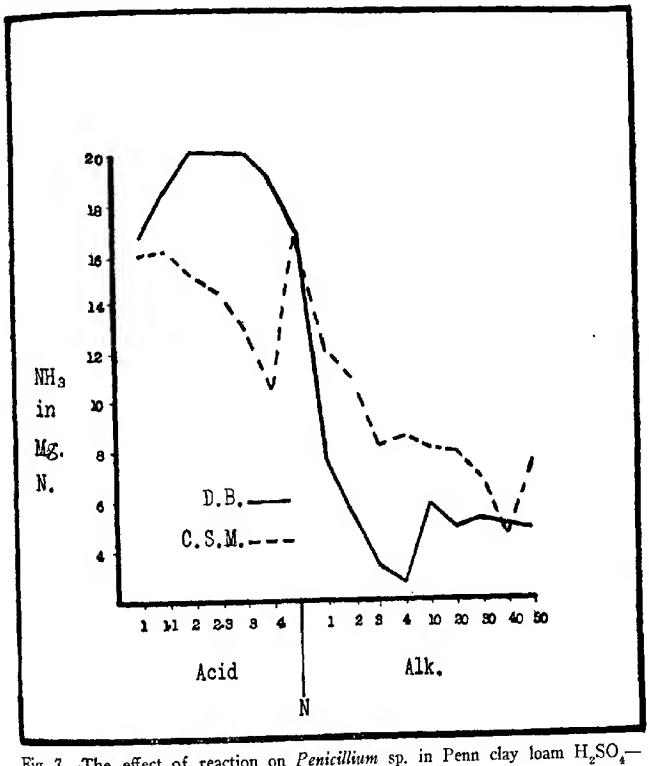


Fig. 7.—The effect of reaction on *Penicillium* sp. in Penn clay loam (H_2SO_4—$CaCO_3$).

The effect of reaction on ammonification by *Zygorrhyncus Vuilleminii* in Penn clay loam using dried blood are given in Table XIII and figure 9. It is evident that there is an increase in ammonia with an increase in acidity from the neutral point to 2,300 pounds. There is a decrease where an acidity of 3,000 pounds obtains, but this is not continued in the case of 4,000 pounds. It is difficult to account for this singular exception. In-

TABLE X

THE EFFECT OF REACTION ON PENICILLIUM SP. IN PENN CLAY LOAM
(H_2SO_4—$CaCO_3$)

No.	Organic Matter	Treatment	NH_3 accumulated in			Increase over check
			Mg. N.	Mg. N.	Av. Mg. N.	Mg. N.
	155 mg. N. Cottonseed Meal	Check	3.32	3.08	3.20	.....
93-94	"	Acid ⇔ 1000 lbs. CaO....	19.18	19.32	19.25	16.05
95-96	"	Soil L. R. ⇔ 1100 lbs. CaO	19.40	19.40	19.40	16.20
97-98	"	Acid ⇔ 2000 lbs. CaO....	18.46	18.93	18.70	15.50
99-100	"	Acid ⇔ 2300 lbs. CaO....	17.80	17.76	17.78	14.58
101-102	"	Acid ⇔ 3000 lbs. CaO....	16.65	15.80	16.23	13.03
103-104	"	Acid ⇔ 4000 lbs. CaO....	14.35	13.50	13.93	10.73
105-106	"	Neutral	19.72	20.55	20.14	16.94
107-108	"	Alk. ⇔ 1000 lbs CaO.....	15.40	15.12	15.26	12.06
109-110	"	Alk. ⇔ 2000 lbs. CaO.....	14.70	14.00	14.35	11.15
111-112	"	Alk. ⇔ 3000 lbs. CaO.....	11.48	11.48	11.48	8.28
113-114	"	Alk ⇔ 4000 lbs. CaO.....	11.20	13.44	12.32	9.12
115-116	"	Alk. ⇔ 10,000 lbs. CaO...	11.47	11.40	11.44	8.24
117-118	"	Alk. ⇔ 20,000 lbs. CaO...	11.10	11.40	11.25	8.05
119-120	"	Alk. ⇔ 30,000 lbs. CaO...	10.80	10.20	10.50	7.30
121-122	"	Alk. ⇔ 40,000 lbs. CaO...	7.98	Lost	7.98	4.78
123-124	"	Alk. ⇔ 50,000 lbs. CaO...	11.60	10.05	10.83	7.63

TABLE XI

THE EFFECT OF REACTION ON ZYGORRHYNCUS VUILLEMINII IN NORFOLK SANDY LOAM
(H_2SO_4—$CaCO_3$)

No.	Organic Matter	Treatment	NH_3 accumulated in			Increase over check
			Mg. N.	Mg. N.	Av. Mg. N.	Mg. N.
	155 mg. N. Dried Bl'd	Check	2.53	2.27	2.40	.....
125-126	"	Acid ⇔ 1000 lbs. CaO....	5.39	5.41	5.40	3.00
127-128	"	Acid ⇔ 2000 lbs. CaO....	10.74	10.68	10.71	8.31
129-130	"	Soil L. R. ⇔ 2300 lbs. CaO	7.28	9.10	8.19	5.79
131-132	"	Acid ⇔ 3000 lbs. CaO....	3.95	4.55	4.25	1.85
133-134	"	Acid ⇔ 4000 lbs. CaO....	3.78	6.72	5.25	2.85
135-136	"	Neutral	2.95	3.00	2.98	0.58
137-138	"	Alk. ⇔ 1000 lbs. CaO.....	2.80	2.57	2.69	0.29
139-140	"	Alk. ⇔ 2000 lbs. CaO.....	3.39	3.30	3.35	0.95
141-142	"	Alk. ⇔ 3000 lbs. CaO.....	2.80	2.81	2.81	0.41
143-144	"	Alk. ⇔ 4000 lbs. CaO.....	2.75	2.50	2.63	0.23
145-146	"	Alk. ⇔ 10,000 lbs. CaO...	4.34	4.34	4.34	1.94
147-148	"	Alk. ⇔ 20,000 lbs. CaO...	3.29	3.92	3.61	1.21
149-150	"	Alk. ⇔ 30,000 lbs. CaO...	3.78	3.71	3.75	1.35
151-152	"	Alk. ⇔ 40,000 lbs. CaO...	3.92	3.78	3.85	1.45
153-154	"	Alk. ⇔ 50,000 lbs. CaO...	3.50	3.06	3.28	0.88

creasing the alkalinity up to 4,000 pounds reduced the ammonia accumulation to such a degree as to make differences in treatment insignificant. However, the phenomenon previously noted, namely the slight increase in ammonia with large applications of $CaCO_3$ is again apparent.

In the cottonseed meal series shown in Table XIV and figure 9, the variations in that part of the experiment dealing with acidity do not permit of any definite conclusion, other than that an increase in acidity beyond 3,000 pounds causes a decreased ammonia accumulation. With increased alkalinity up to 10,000 pounds there is a gradual decrease in ammonia. Additions of $CaCO_3$ beyond this point produce no differences worthy of note.

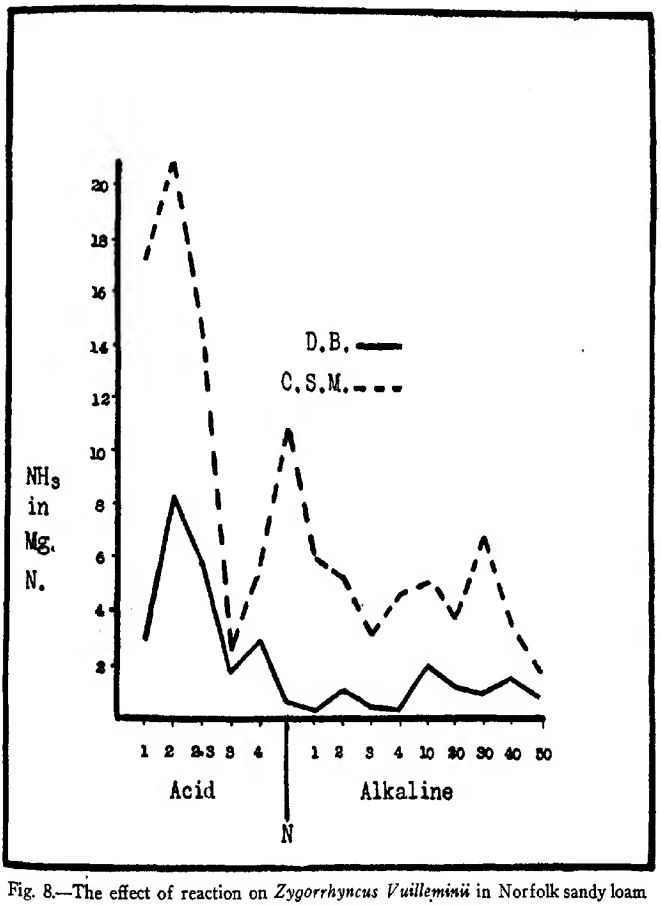


Fig. 8.—The effect of reaction on *Zygorrhyncus Vuilleminii* in Norfolk sandy loam (H_2SO_4—$CaCO_3$).

In general, while the results concerning the effect of reaction on ammonification indicate clearly that in sandy soil with both kinds of or-

TABLE XII

THE EFFECT OF REACTION ON ZYGORRHYNCUS VUILLEMINII IN NORFOLK SANDY LOAM
(H_2SO_4—$CaCO_3$)

No.	Organic Matter	Treatment	NH_3 accumulated in			Increase over check
			Mg. N.	Mg. N.	Av. Mg. N.	Mg. N.
	155 mg. N. Cottonseed Meal	Check	2.91	2.69	2.80	.....
155-156	"	Acid ⇔ 1000 lbs. CaO....	19.80	20.20	20.00	17.20
157-158	"	Acid ⇔ 2000 lbs. CaO....	23.80	23.72	23.72	20.92
159-160	"	Soil L. R. ⇔ 2300 lbs. CaO	17.70	18.22	17.96	14.16
161-162	"	Acid ⇔ 3000 lbs. CaO....	4.90	5.88	5.39	2.59
163-164	"	Acid ⇔ 4000 lbs. CaO....	7.49	9.10	8.30	5.50
165-166	"	Neutral	13.70	13.57	13.64	10.84
167-168	"	Alk. ⇔ 1000 lbs. CaO.....	8.98	8.80	8.89	6.09
169-170	"	Alk. ⇔ 2000 lbs. CaO.....	8.40	7.90	8.15	5.35
171-172	"	Alk. ⇔ 3000 lbs. CaO.....	6.40	6.40	6.40	3.60
173-174	"	Alk. ⇔ 4000 lbs. CaO.....	7.90	7.07	7.49	4.69
175-176	"	Alk. ⇔ 10,000 lbs. CaO...	9.16	6.78	7.97	5.17
177-178	"	Alk. ⇔ 20,000 lbs. CaO...	6.29	7.06	6.67	3.87
179-180	"	Alk. ⇔ 30,000 lbs. CaO...	9.58	9.58	9.58	6.78
181-182	"	Alk. ⇔ 40,000 lbs. CaO...	6.78	5.80	6.29	3.49
183-184	"	Alk. ⇔ 50,000 lbs. CaO...	5.97	3.31	4.64	1.84

TABLE XIII

THE EFFECT OF REACTION ON ZYGORRHYNCUS VUILLEMINII IN PENN CLAY LOAM
(H_2SO_4—$CaCO_3$)

No.	Organic Matter	Treatment	NH_3 accumulated in			Increase over check
			Mg. N.	Mg. N.	Av. Mg. N.	Mg. N.
	155 mg. N. Dried Bl'd	Check	2.73	2.87	2.80	.....
185-186	"	Acid ⇔ 1000 lbs. CaO....	6.80	6.85	6.83	4.03
187-188	"	Soil L. R. ⇔ 1100 lbs. CaO	6.83	7.42	7.13	4.33
189-190	"	Acid ⇔ 2000 lbs. CaO....	9.38	9.24	9.31	6.51
191-192	"	Acid ⇔ 2300 lbs. CaO....	8.89	10.64	9.77	6.97
193-194	"	Acid ⇔ 3000 lbs. CaO....	9.66	7.91	8.79	5.99
195-196	"	Acid ⇔ 4000 lbs. CaO....	10.08	10.50	10.29	7.49
197-198	"	Neutral	5.88	6.30	6.09	3.29
199-200	"	Alk. ⇔ 1000 lbs. CaO.....	4.00	4.00	4.00	1.20
201-202	"	Alk. ⇔ 2000 lbs. CaO.....	3.99	4.24	4.12	1.32
203-204	"	Alk. ⇔ 3000 lbs. CaO.....	3.45	3.36	3.41	0.61
205-206	"	Alk. ⇔ 4000 lbs. CaO.....	3.50	3.60	3.55	0.75
207-208	"	Alk. ⇔ 10,000 lbs. CaO...	4.90	4.90	4.90	2.10
209-210	"	Alk. ⇔ 20,000 lbs. CaO...	6.09	4.20	5.15	2.35
211-212	"	Alk. ⇔ 30,000 lbs. CaO...	5.60	5.04	5.32	2.52
213-214	"	Alk. ⇔ 40,000 lbs. CaO...	4.34	4.34	4.34	1.54
215-216	"	Alk. ⇔ 50,000 lbs. CaO...	4.62	4.48	4.55	1.75

ganic matter, an increase in acidity from the neutral point to 2,000 pounds causes a corresponding increase in ammonia accumulation. Above the latter point there appears to be a decrease in ammonia. In clay soil a slightly greater acidity, 2,300 to 3,000 pounds produces the maximum ammonia accumulation. It is possible to suppose that the reason

for the fact that a greater acidity is necessary for maximum ammonia accumulation in clay than in sandy soil, lies in the fact that the distribution of any acid would be more thorough in the case of the sandy soil and consequently a smaller amount would be required. In both soils a further increase in acidity was responsible for a decrease in ammonia. An increase in alkalinity from 1,000 to 4,000 pounds was responsible, generally speaking, for a diminution of ammonia; while applications from 10,000 to 50,000 pounds of CaO per acre yielded approximately a constant quantity of ammonia.

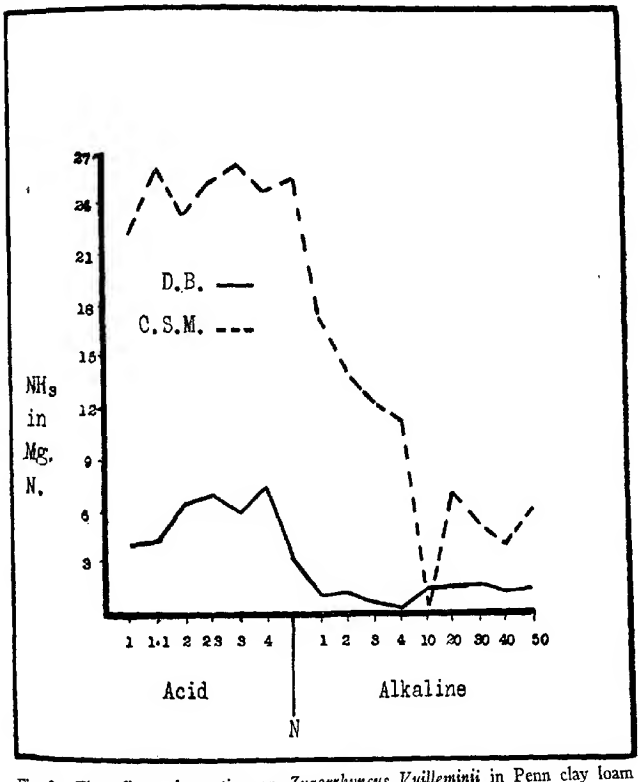


Fig. 9.—The effect of reaction on *Zygorrhyncus Vuilleminii* in Penn clay loam (H_2SO_4—$CaCO_3$).

In Table XV and figure 10 are shown the effect of reaction on ammonification by *Rhizopus nigricans* (using 36,000 spores per 1 c.c.) in Norfolk sandy loam with dried blood as the source of organic matter. In increasing the acidity from the neutral point to 2,000 pounds there is a

TABLE XIV

THE EFFECT OF REACTION ON ZYGORRHYNCUS VUILLEMINII IN PENN CLAY LOAM

(H_2SO_4—$CaCO_3$)

No.	Organic Matter	Treatment	NH_3 accumulated in			Increase over check
			Mg. N.	Mg. N.	Av. Mg. N.	Mg. N.
	155 mg. N. Cottonseed Meal	Check	3.32	3.08	3.20	.....
217-218	"	Acid ⇔ 1000 lbs. CaO....	26.01	25.40	25.71	22.51
219-220	"	Soil L. R. ⇔ 1100 lbs. CaO	29.12	29.26	29.19	25.99
221-222	"	Acid ⇔ 2000 lbs. CaO....	26.82	26.32	26.57	23.37
223-224	"	Acid ⇔ 2300 lbs. CaO....	27.16	29.89	28.53	25.33
225-226	"	Acid ⇔ 3000 lbs. CaO....	30.66	29.61	30.14	26.94
227-228	"	Acid ⇔ 4000 lbs. CaO....	28.00	28.00	28.00	24.80
229-230	"	Neutral	28.14	29.47	28.81	25.61
231-232	"	Alk. ⇔ 1000 lbs. CaO.....	21.08	20.91	21.00	17.80
233-234	"	Alk. ⇔ 2000 lbs. CaO.....	17.25	17.53	17.39	14.19
235-236	"	Alk. ⇔ 3000 lbs. CaO.....	15.60	15.79	15.70	12.50
237-238	"	Alk. ⇔ 4000 lbs. CaO.....	14.87	14.60	14.74	11.54
239-240	"	Alk. ⇔ 10,000 lbs. CaO...	3.06	3.99	3.53	0.33
241-242	"	Alk. ⇔ 20,000 lbs. CaO...	10.43	10.08	10.26	7.06
243-244	"	Alk. ⇔ 30,000 lbs. CaO...	8.05	9.10	8.53	5.33
245-246	"	Alk. ⇔ 40,000 lbs. CaO...	7.21	7.49	7.35	4.15
247-248	"	Alk. ⇔ 50,000 lbs. CaO...	9.38	Lost	9.38	6.18

TABLE XV

THE EFFECT OF REACTION ON RHIZOPUS NIGRICANS IN NORFOLK SANDY LOAM

(H_2SO_4—$CaCO_3$)

No.	Organic Matter	Treatment	NH_3 accumulated in			Increase over check
			Mg. N.	Mg. N.	Av. Mg. N.	Mg. N.
	155 mg. N. Dried Bl'd	Check	2.53	2.27	2.40	.....
249-250	"	Acid ⇔ 1000 lbs. CaO....	19.32	17.74	18.53	16.13
251-252	"	Acid ⇔ 2000 lbs. CaO....	28.57	28.47	28.52	26.12
253-254	"	Soil L. R. ⇔ 2300 lbs. CaO	13.16	14.49	13.83	11.43
255-256	"	Acid ⇔ 3000 lbs. CaO....	10.22	12.88	11.55	9.15
257-258	"	Acid ⇔ 4000 lbs. CaO....	8.75	9.52	9.14	6.74
259-260	"	Neutral	11.80	12.70	12.25	9.85
261-262	"	Alk. ⇔ 1000 lbs. CaO.....	8.40	9.10	8.75	6.35
263-264	"	Alk. ⇔ 2000 lbs. CaO.....	5.20	5.70	5.45	3.05
265-266	"	Alk. ⇔ 3000 lbs. CaO.....	7.30	5.25	6.78	4.38
267-268	"	Alk. ⇔ 4000 lbs. CaO.....	4.55	4.87	4.76	2.36
269-270	"	Alk. ⇔ 10,000 lbs. CaO...	4.69	7.84	6.27	3.87
271-272	"	Alk. ⇔ 20,000 lbs. CaO...	9.38	3.85	6.62	4.22
273-274	"	Alk. ⇔ 30,000 lbs. CaO...	3.85	3.99	3.92	1.52
275-276	"	Alk. ⇔ 40,000 lbs. CaO...	1.89	3.36	2.63	0.23
277-278	"	Alk. ⇔ 50,000 lbs. CaO...	3.92	3.99	3.96	1.56

gradual and pronounced increase in ammonia. In the cottonseed meal series as shown in Table XVI and figure 10, the maximum ammonia accumulation (with but one exception) occurs between the neutral point and an acidity of 2,300 pounds. Above the latter point there is a decrease in ammonia with increasing acidity. From the neutral point to 4,000 pounds alkalinity there is a gradual decrease in ammonia (with one

exception) with a corresponding increase in alkalinity. From 10,000 to 50,000 pounds CaO there is too great a variation in the results to permit more than the assertion that there appears to be a tendency toward a decrease in ammonia with increasing alkalinity. McLean and Wilson (14) working with *Rhizopus nigricans* in a gravelly loam soil having a lime requirement of 1,200 pounds CaO per acre and using 155 mg. N in cottonseed meal found that the addition of 0.5 per cent and 2 per cent $CaCO_3$ did not greatly affect ammonia production, though there was a slight tendency towards an increase. Naturally enough the organism employed by these investigators in all likelihood was of a different strain

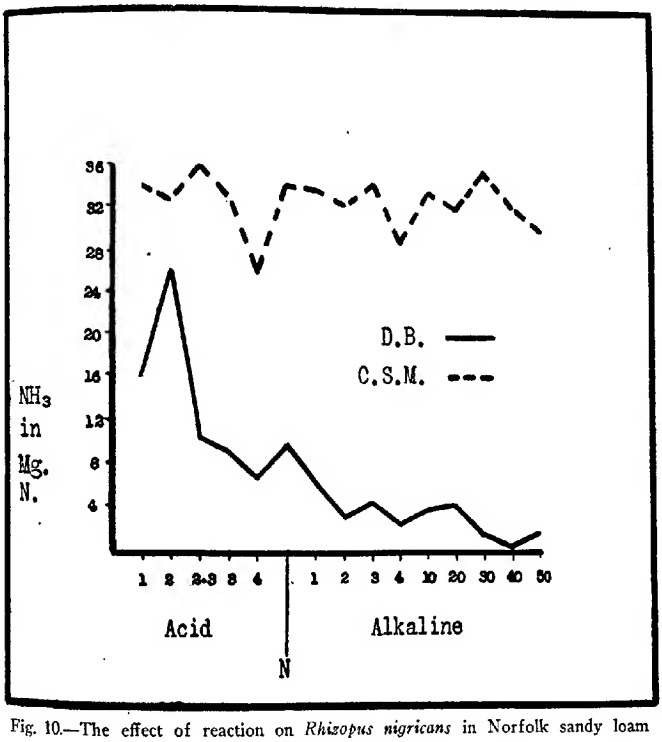


Fig. 10.—The effect of reaction on *Rhizopus nigricans* in Norfolk sandy loam (H_2SO_4—$CaCO_3$).

from that used in the present experiments and undoubtedly this would account for the divergence in results. The data obtained by McLean and Wilson (14) and those recorded in Table XVI are in agreement in showing that *Rhizopus nigricans* attains its maximum ammonia accumulation in a sandy soil having a neutral reaction. However, when the alkalinity

TABLE XVI

THE EFFECT OF REACTION ON RHIZOPUS NIGRICANS IN NORFOLK SANDY LOAM (H_2SO_4—$CaCO_3$)

No.	Organic Matter	Treatment	NH_3 accumulated in			Increase over check Mg. N.
			Mg. N.	Mg. N.	Av. Mg. N.	
	155 mg. N. Cottonseed Meal	Check	2.91	2.69	2.80	.....
279-280	"	Acid ⇔ 1000 lbs. CaO....	36.53	37.08	36.81	34.01
281-282	"	Acid ⇔ 2000 lbs. CaO....	35.57	35.55	35.56	32.76
283-284	"	Soil L. R. ⇔ 2300 lbs. CaO	39.27	38.22	38.75	35.95
285-286	"	Acid ⇔ 3000 lbs. CaO....	37.24	35.14	36.19	33.39
287-288	"	Acid ⇔ 4000 lbs. CaO....	28.56	29.12	28.84	26.04
289-290	"	Neutral	36.60	37.04	36.87	34.07
291-292	"	Alk. ⇔ 1000 lbs. CaO.....	35.88	36.83	36.36	33.56
293-294	"	Alk. ⇔ 2000 lbs. CaO.....	36.46	33.75	35.11	32.31
295-296	"	Alk. ⇔ 3000 lbs. CaO.....	36.82	39.34	37.08	34.28
297-298	"	Alk. ⇔ 4000 lbs. CaO.....	31.55	31.45	31.50	28.70
299-300	"	Alk. ⇔ 10,000 lbs. CaO...	35.42	36.68	36.05	33.25
301-302	"	Alk. ⇔ 20,000 lbs. CaO...	34.72	34.72	34.72	31.92
303-304	"	Alk. ⇔ 30,000 lbs. CaO...	42.81	35.14	38.98	36.18
305-306	"	Alk. ⇔ 40,000 lbs. CaO...	36.68	32.90	34.79	31.99
307-308	"	Alk. ⇔ 50,000 lbs. CaO...	32.20	33.04	32.62	29.82

TABLE XVII

THE EFFECT OF REACTION ON RHIZOPUS NIGRICANS IN PENN CLAY LOAM (H_2SO_4—$CaCO_3$)

No.	Organic Matter	Treatment	NH_3 accumulated in			Increase over check Mg. N.
			Mg. N.	Mg. N.	Av. Mg. N.	
	155 mg. N. Dried Bl'd	Check	2.73	2.87	2.80	.....
309-310	"	Acid ⇔ 1000 lbs. CaO....	11.30	11.90	11.60	8.80
311-312	"	Soil L. R. ⇔ 1100 lbs. CaO	11.06	11.20	11.13	8.33
313-314	"	Acid ⇔ 2000 lbs. CaO....	10.22	10.57	10.39	7.59
315-316	"	Acid ⇔ 2300 lbs. CaO....	10.78	9.80	10.29	7.49
317-318	"	Acid ⇔ 3000 lbs. CaO....	11.20	11.20	11.20	8.40
319-320	"	Acid ⇔ 4000 lbs. CaO....	9.66	9.17	9.41	6.61
321-322	"	Neutral	9.87	10.95	10.41	7.61
323-324	"	Alk. ⇔ 1000 lbs. CaO.....	8.90	9.95	9.43	6.63
325-326	"	Alk. ⇔ 2000 lbs. CaO.....	6.33	6.43	6.38	3.58
327-328	"	Alk. ⇔ 3000 lbs. CaO.....	4.81	4.97	4.89	2.09
329-330	"	Alk. ⇔ 4000 lbs. CaO.....	4.25	3.85	4.05	1.25
331-332	"	Alk. ⇔ 10,000 lbs. CaO...	6.73	6.51	6.62	3.82
333-334	"	Alk. ⇔ 20,000 lbs. CaO...	5.74	6.44	6.09	3.19
335-336	"	Alk. ⇔ 30,000 lbs. CaO...	6.02	6.02	6.02	3.22
337-338	"	Alk. ⇔ 40,000 lbs. CaO...	6.39	6.35	6.37	3.57
339-340	"	Alk. ⇔ 50,000 lbs. CaO...	6.72	5.46	6.09	3.29

is increased beyond this point they find a tendency toward increased ammonia accumulation where the present work indicates that the general trend is towards a decrease in ammonia.

The effect of soil reaction on ammonification by *Rhizopus nigricans* in Penn clay loam, using dried blood is shown in Table XVII and figure 11. The maximum ammonification occurs between the neutral point and an acidity of 3,000 pounds, the differences between treatments being in-

significant. With an acidity of 4,000 pounds there is a decrease in ammonia. It is to be noted that in this experiment the number of spores used for inoculation was so small (32,000 per 1 c.c.) that the ammonia accumulation was comparatively slight, consequently the differences in treatment could hardly have been expected to be very striking. However, it is quite clearly demonstrated that with increasing alkalinity up to 4,000 pounds there is a corresponding decrease in ammonia. When the alkalinity is increased beyond this point, the phenomenon already referred to, namely a constant, makes its appearance.

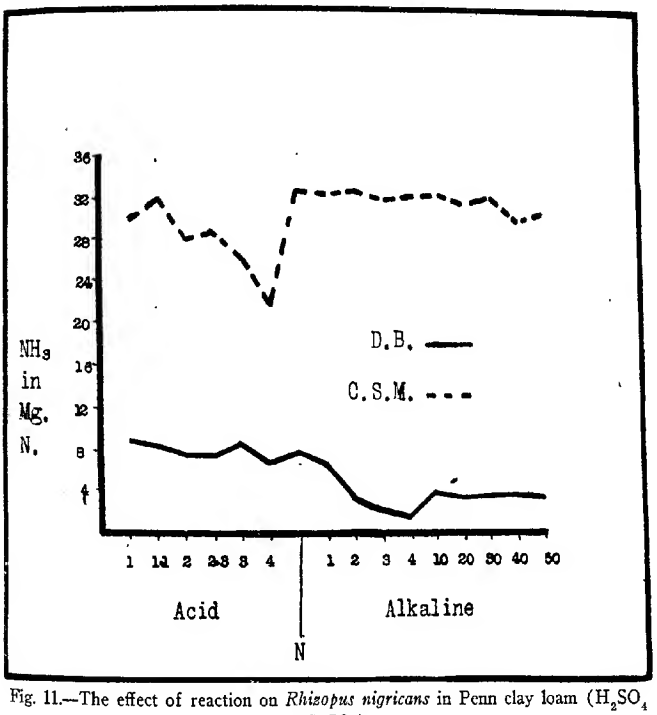


Fig. 11.—The effect of reaction on *Rhizopus nigricans* in Penn clay loam (H_2SO_4—$CaCO_3$).

In the cottonseed meal series as shown in Table XVIII and figure 11, the maximum ammonia accumulation occurs between the neutral point and an acidity of 1,100 pounds. With 2,000 and 2,300 pounds the amount of ammonia is somewhat lower, but with an increase in acidity beyond this point there is a corresponding decrease in ammonia. The addition of various amounts of $CaCO_3$ in this instance resulted in practically no differences in ammonia accumulation. An explanation of this phenomenon

might depend upon the fact that in such a heavy soil, $CaCO_3$ was not able to act sufficiently rapidly to overcome the acidity of the soil together with that produced by the by-products of the decomposition of cottonseed meal.

TABLE XVIII

THE EFFECT OF REACTION ON RHIZOPUS NIGRICANS IN PENN CLAY LOAM (H_2SO_4—$CaCO_3$)

No.	Organic Matter	Treatment	NH_3 accumulated in			Increase over check
			Mg. N.	Mg. N.	Av. Mg. N.	Mg. N.
	155 mg. N. Cottonseed Meal	Check	3.32	3.08	3.20	.....
341-342	"	Acid = 1000 lbs. CaO....	33.69	33.33	33.51	30.31
343-344	"	Soil L. R. = 1100 lbs. CaO	35.70	34.58	35.14	31.94
345-346	"	Acid = 2000 lbs. CaO....	30.80	31.76	31.28	28.08
347-348	"	Acid = 2300 lbs. CaO....	31.36	32.06	31.71	28.51
349-350	"	Acid = 3000 lbs. CaO....	29.68	28.70	29.19	25.99
351-352	"	Acid = 4000 lbs. CaO....	23.91	25.90	24.95	21.75
353-354	"	Neutral	37.10	36.96	37.03	33.83
355-356	"	Alk. = 1000 lbs. CaO.....	35.43	35.33	35.38	32.18
357-358	"	Alk. = 2000 lbs. CaO.....	35.54	36.18	35.86	32.66
359-360	"	Alk. = 3000 lbs. CaO.....	35.86	34.84	35.35	32.15
361-362	"	Alk. = 4000 lbs. CaO.....	35.18	35.58	35.38	32.18
363-364	"	Alk. = 10,000 lbs. CaO...	36.54	35.14	35.84	32.64
365-366	"	Alk. = 20,000 lbs. CaO...	34.86	Lost	34.86	31.66
367-368	"	Alk. = 30,000 lbs. CaO...	36.68	33.88	35.28	32.08
369-370	"	Alk. = 40,000 lbs. CaO...	33.46	32.76	33.11	29.91
371-372	"	Alk. = 50,000 lbs. CaO...	34.58	33.39	33.99	30.79

Again, the facts cited above have the same general tendency as those previously referred to (14). For in the latter case using the same kind of soil as in the present instance (except for the fact that it was neutral), it was found that a high increase in alkalinity caused a depression in ammonia accumulation. Where dried blood was used instead of cottonseed meal, as a source of organic matter, the results were likewise in agreement. It is also of interest to note that the above investigators found that *Trichoderma Koningi* evidently requires a neutral medium for its best growth.

Considering the data which have been presented concerning the effect of reaction on ammonification by *Rhizopus nigricans* using both organic materials in sandy soil, the maximum ammonia accumulation takes place with a reaction between the neutral point and 2,300 pounds, while in Penn clay loam, due no doubt to its physical condition, an acidity of 3,000 pounds would represent the outer limit of maximum ammonia accumulation. In general, also, it may be stated (subject to the exceptions already noted) that an increase in alkalinity from 1,000 to 4,000 pounds CaO per acre causes a diminution in ammonia accumulated.

Considering in their entirety the data which have been presented concerning the effect of soil reaction on ammonification by these fungi where the reaction was altered by additions of H_2SO_4 or $CaCO_3$, the following points are indicated.

1. Alteration of soil reaction has practically the same effect upon the three different fungi studied.

2. The effect of soil reaction is more pronounced where dried blood rather than cottonseed meal is employed as the source of organic nitrogenous matter to be ammonified.

3. The effect of reaction on ammonification by these fungi is more pronounced in clay than in sandy soil.

4. In general the maximum ammonia accumulation by these fungi in sandy or clay soils with either kind of organic matter, occurs between the neutral point and an acidity of 2,000 pounds.

5. An increase in application of $CaCO_3$ causes a diminution in ammonia accumulation.

SUMMARY

Under the conditions of the experiment the following points have been established.

1. *Rhizopus nigricans, Zygorrhyncus Vuilleminii* and *Penicillium* sp. 10 are all influenced in the same way by any specific changes in soil reaction. They possess a comparatively narrow range of reaction tolerance for maximum ammonification which was found to be between the neutral point and an acidity equivalent to 2,000 pounds CaO per acre. In general, an acidity greater than 2,000 pounds caused a depression in ammonification as did an increase in alkalinity beyond the neutral point.

2. It is significant that the results obtained were practically the same whether sandy or clay soils (having either high or low lime requirements) were used with either dried blood or cottonseed meal.

3. Where normal solutions of HCl and NaOH were used to alter soil reaction, the data were somewhat more concordant than where H_2SO_4 and $CaCO_3$ were used for the same purpose.

4. There is good reason then to believe that the practical significance of this experimentation points to the fact that where the soil reaction is unfavorable for the activities of the soil bacteria concerned in ammonification, the soil fungi might prove to be an important compensating factor in maintaining fertility.

In conclusion it is a privilege to express appreciation to Dr. J. G. Lipman for his helpful suggestions ever at the writer's disposal, and to Dr. M. T. Cook and Professor J. P. Helyar for their kind assistance.

LITERATURE CITED

(1) BECK, H.
1902. Einwirkung von Mikroorganismen auf einge chemische Normallösungen. *In* Centbl. Bakt. [etc.], Abt. 1, O., Bd. 32, p. 649.

(2) BUROMSKY, J.
1912. Die Salze Zn, Mg, Ca., K, und Na, und ihre Einflusz auf die Entwicklung von *Aspergillus niger*. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 36, p. 54.

(3) BUTKEWITSCH, W. S.
1903. Um wandlung der Einweisstoffe durch die niederen Pilze, u. s. w. *In* Jahrb. Wiss. Bot., Bd. 38, p. 147.
(4) CLARK, J. F.
1899. The toxic effect of deleterious agents on the germination and development of certain filamentous fungi. *In* Bot. Gaz., v. 28, p. 307-378.
(5) COLEMAN, D. A., LINT, H. C., and KOPELOFF, N.
1916. Can soil be sterilized without radical alteration? *In* Soil Sci., v. 1, no. 3, p. 259-274.
(6) COLEMAN, D. A.
1916. Environmental factors influencing the activities of soil fungi. *In* Soil Sci. (soon to appear).
(7) FAELLI, G.
1904. Richerche di batteriologici agraria nell' agro Romano. *Abs. in* Centbl. Bakt. [etc.], Abt. 2, Bd. 14, p. 423.
(8) FELLERS, C. R.
1916. Some bacteriological studies on agar-agar. *In* Soil Sci. (soon to appear).
(9) FISCHER, H.
1909. Bakteriologisch chemische Untersuchungen. *In* Landw. Jahrb., Bd. 38, p. 358.
(10) HALL, A. D., MILLER, N. H. J., and GIMINGHAM, C. T.
1908. Nitrification in acid soils. *In* Proc. Roy. Soc. [London], Ser. B., v. 80, B 539, p. 196.
(11) KOPELOFF, N.
1916. Inoculation and incubation of soil fungi. *In* Soil Sci., v. 1, no. 4, p. 381-403.
(12) LINT, H. C., and COLEMAN, D. A.
1916. Sources of error in soil bacteriological analyses. *In* Soil Sci. (soon to appear).
(13) LIPMAN, J. G., and BROWN, P. E.
1908. Media for quantitative estimation of soil bacteria. *In* N. J. Agr. Exp. Sta. 29th Ann. Rpt., p. 132-136.
(14) MCLEAN, H. C., and WILSON, G. W.
1914. Ammonification studies with soil fungi. N. J. Agr. Exp. Sta. Bul. 270, 39 p., 1 pl.
(15) MARCHAL, E.
1893. The production of ammonia in the soil by microbes. *In* Bul. Acad. Sci. Belgique, (3) v. 25, p 741.
(16) OUDEMANS, C. A. J. A., and KONING, C. J.
1902. Prodrome d'une flore mycologique obtenue par la culture sur gélatine prézarée de la terre humeuse du Spanderswood prés de Bussum. *In* Arch. Néerland. Sci. Exact. et Nat., s. 2, t. 7, p. 266.
(17) PLANCHON, L.
1900. The effect of chemical media on the growth of fungi. *In* Ann. Sci. Nat. Bot., s. 8, t. 11, p. 1-248.
(18) RAMANN, E.
1911. Bodenkunde. 3rd Aufl., p. 432.

(19) ROBERT, MLLE.
1911. The influence of calcium on the development and ash constituents of *Aspergillus niger*. *In* Compt. Rend. Acad. Sci. [Paris], t. 153, no. 23, p. 1175-1177.

(20) SZUTON, M. B.
1912. The comparative influence of potassium, rubidium and caesium on the development and sporulation of *Aspergillus niger*. *In* Compt. Rend. Acad. Sci. [Paris], t. 155, p. 1181-83.

(21) STEVENS, F. L.
1898. The effect of aqueous solutions upon the germination of fungous spores. *In* Bot. Gaz., v. 26, p. 377.

(22) THOM, C.
1910. The effects of acidity of culture media on morphology in species of *Penicillium*. *In* Science, n. s., v. 31, no. 799, p. 635.

(23) TRAAEN, A. E.
1915. Untersuchungen über Bodenpilze aus Norwegen. *In* Centbl. Agr. Chem., Bd. 44, no. 1, p. 347.

(24) WAKSMAN, S. A.
1916. Fungi and their activities in the soil. *In* Soil Sci. (soon to appear.)

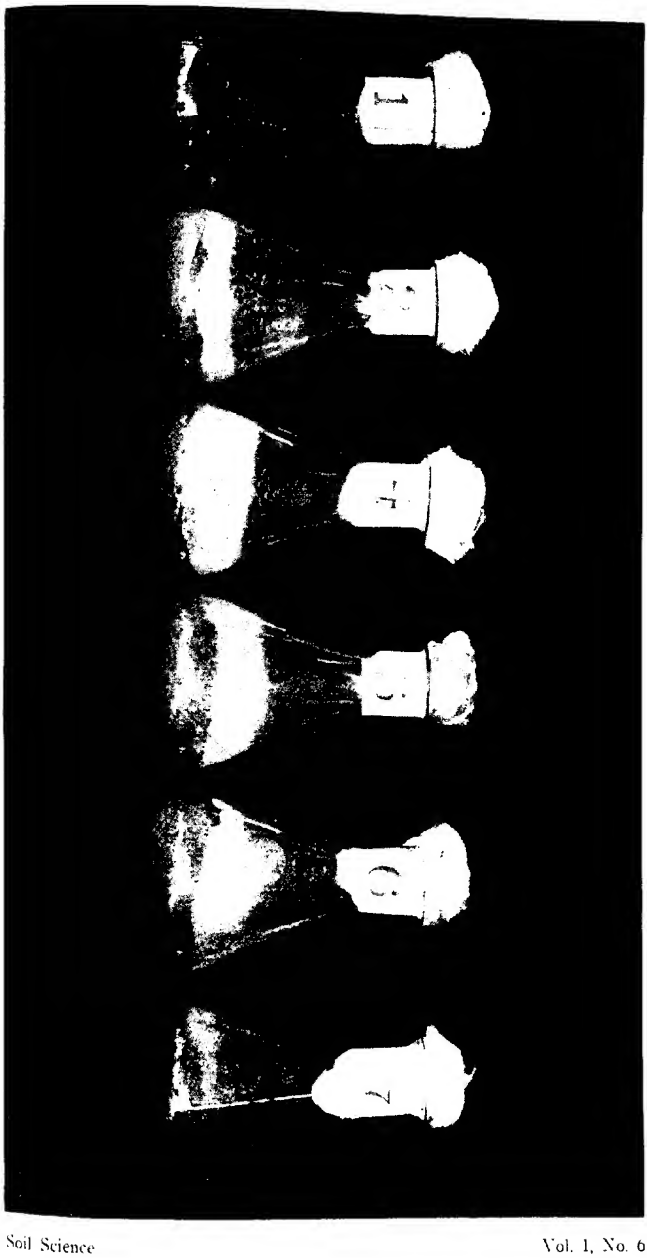
(25) WINOGRADSKY, S.
1884. Uber die Wirkung äusserer Einflüsse auf die Entwicklung von Mycorderma vini. *In* Arb. d. St. Petersb. naturf. Ges., Bd. 14, p. 132. Abs. Bot. Centbl., Bd. 20 (1884), p. 165.

PLATE I

The effect of soil reaction on mycelial growth of *Rhizopus nigricans* using dried blood as the source of organic matter.

HCl(N/1) added in amounts equivalent to:

1. Check.
2. Original soil acid = 400 lbs. CaO per acre.
4. Acid = 1000 lbs.
5. Acid = 2000 lbs.
6. Acid = 3000 lbs.
7. Acid = 4000 lbs.



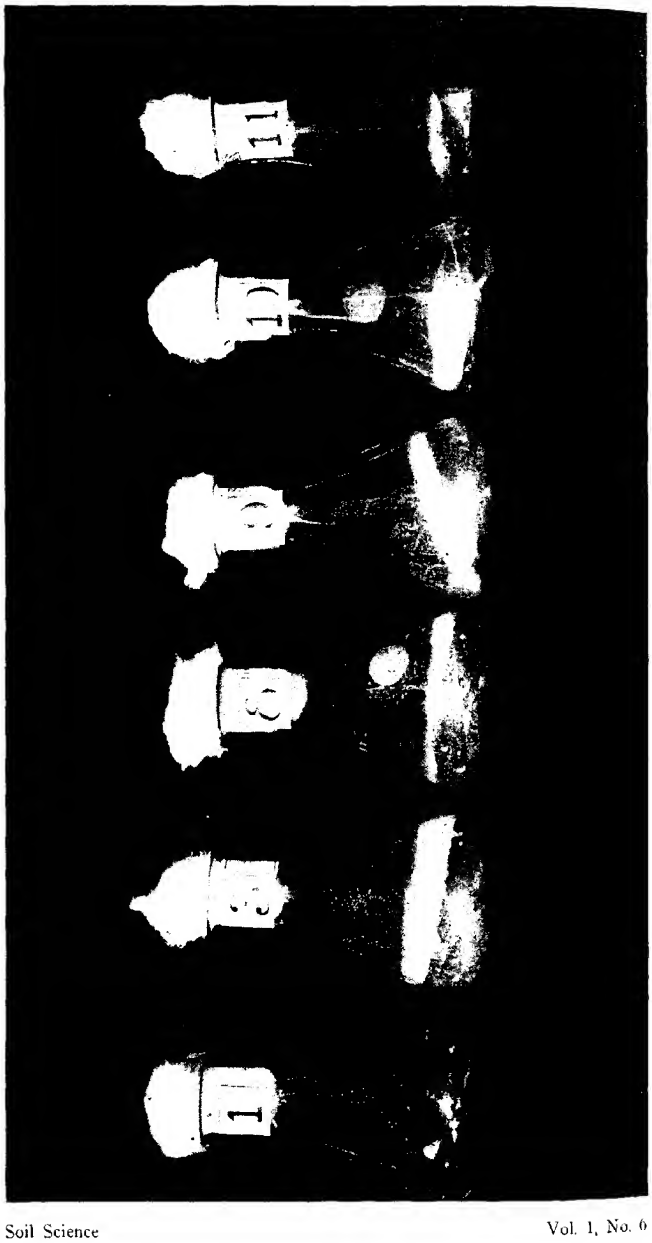

11
10
9
8
7
1

PLATE II

The effect of soil reaction on mycelial growth of *Rhizopus nigricans* using dried blood as the source of organic matter.

NaOH(N/1) added in amounts equivalent to:

1. Check.
2. Neutral.
8. Alk. $\simeq$ 1000 lbs. CaO per acre.
9. Alk. $\simeq$ 2000 lbs.
10. Alk. $\simeq$ 3000 lbs.
11. Alk. $\simeq$ 4000 lbs.

PLATE III

The effect of soil reaction on mycelial growth of *Rhizopus nigricans* using cotton-seed meal as the source of organic matter.

HCl(N/1) added in amounts equivalent to:

1. Check.
2. Original soil acid $\simeq$ 400 lbs. CaO per acre.
4. Acid $\simeq$ 1000 lbs.
5. Acid $\simeq$ 2000 lbs.
6. Acid $\simeq$ 3000 lbs.
7. Acid $\simeq$ 4000 lbs.

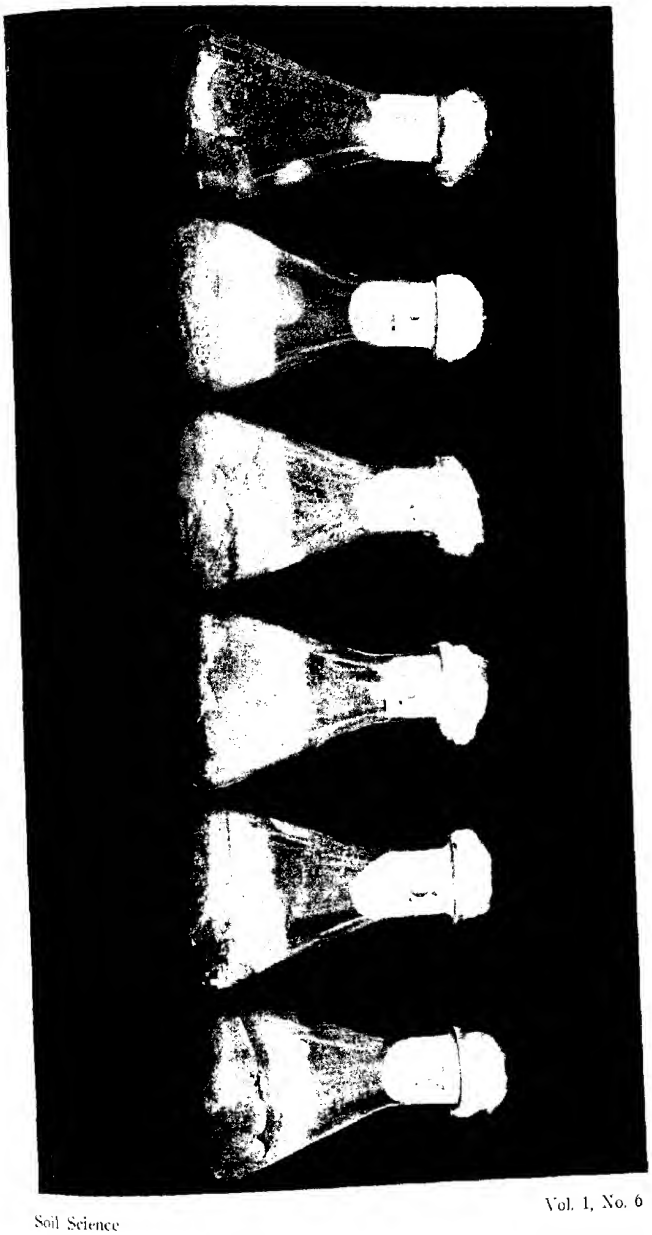


PLATE IV

The effect of soil reaction on mycelial growth of *Rhizopus nigricans* using cotton-seed meal as the source of organic matter.

NaOH(N/1) added in amounts equivalent to:

1. Check.
3. Neutral.
8. Alk. $\simeq$ 1000 lbs. CaO per acre.
9. Alk. $\simeq$ 2000 lbs.
10. Alk. $\simeq$ 3000 lbs.
11. Alk. $\simeq$ 4000 lbs.

ACCUMULATION OF SALTS IN OHIO SOILS[1]

By

J. W. AMES, *Chemist*, and C. J. SCHOLLENBERGER, *Assistant Chemist*,
Ohio Agricultural Experiment Station

This phenomenon is not of common occurrence in soils of the humid regions where the rainfall is sufficiently distributed throughout the year to enable the gravitational water to sweep downward any excessive amount of soluble minerals which have been carried toward the surface by capillary water. While the accumulation of soluble salts at or near the surface forming alkali deposits is common in semi-arid and arid sections, no similar occurrences in humid soils have been reported so far as we know, except by Cameron.[2] He reports alkali spots observed by Dr. Whitney at the Maryland Experiment Station and near Starke, Bradford County, Florida. The formation in Maryland contains about 2 per cent of water-soluble salts. About 50 per cent of the material was calcium nitrate and 90 per cent was in the form of nitrates.

The crust found in Florida was composed chiefly of sodium chloride; sulphates and phosphates were also present in measurable quantities. Accumulations consisting chiefly of sodium chloride were reported in Mississippi, Louisiana and Texas. Deposits of soluble sulphates which were considered to be due to the oxidation of iron sulphide are also reported as having been found in Maryland and New York.

CASES OBSERVED IN OHIO

So far as they have been observed, the areas in Ohio affected in this way are located in the southern part of Highland County and in Brown and Clermont Counties where the loess soils overlie the Illinois glaciation. The underlying rock in this section is limestone which is covered by from 10 to 25 feet of boulder clay. Leverett[3] reports that occasional exposures of residual clays between the blue till and the rock are found.

[1] Received for publication May 11, 1916.

[2] Cameron, F. C. Soil solutions. U. S. Dept. Agr. Bur. Soils, Bul. 17, 39 p., 1901.

[3] Leverett, F. Glacial formations and drainage features of the Erie and Ohio Basins. U. S. Geol. Survey Monograph 41, 1902.

Orton[1] states that occasionally black, mucky clay lies immediately below the blue till and a few feet above the rock. In this same report reference is made to a deposit of soil and bog iron ore between the yellow and blue till which is said to extend over an area of several miles. The information available concerning the geology of this section does not furnish any explanation as to the source of the excess of salts.

Where this condition was found the deposition of soluble salts carried to the surface by capillary waters forms a noticeable effloresence resembling frost on the soil. The excessive salt concentration of the soil water is also indicated by a white coating on the sparse vegetation, mostly weeds, growing on these spots.

The soils on which the deposits were observed are very poorly drained. Indications of the excess of water were seen in the numerous workings of crayfish found in the locality. It is stated by the owners of land where these formations occur that they are most pronounced after a heavy rainfall. This indicates that the subsoil water is strongly impregnated with salts, for when a connection is established between the saline subsoil water and the water evaporating from the surface, a capillary rise of salts takes place followed by a crystallization at the surface.

The Salt Content of Well-Water

The fact that the water of shallow wells which are from 8 to 12 feet deep is strongly impregnated with salts of calcium and magnesium also furnishes further evidence that the subsoil water holds excessive amounts of these salts in solution. The water from a shallow well about one mile from one of the soils examined contained 3.59 gm. total solids per liter.[1] The amounts of calcium, magnesium and sulphate found were equivalent to 1.65 gm. of calcium sulphate ($CaSO_4 2H_2O$) and 3.76 gm. magnesium sulphate ($MgSO_4 7H_2O$). No aluminum or iron was found in the water. Calcium sulphide is reported in artesian water at Ripley, Brown County, as 14.9 grains. This water also contains calcium hyposulphite 2.58 grains per gallon.[2]

Composition of Salt Formation

Water extracts of one of the soils on which deposits of salts were observed and of adjacent soil which appeared to be free from salts were obtained by extracting 50 gm. of the surface soil with 1000 c.c. of water. The results expressed as per cent in soil show that magnesium sulphate and aluminum sulphate were the chief constituents; only a slight trace of calcium was found.

[1] Orton, E. Geology of Clermont County. *In* Rpt. Geol. Survey Ohio, v. 1 p. 443, 1875.

[2] Leverett, F. Water resources of Indiana and Ohio. *In* U. S. Geol. Survey, 18th Ann. Rpt., pt. 4, p. 496, 1897.

The magnesium, aluminum and sulphate found are equivalent to 4.27 per cent $MgSO_4 \cdot 7H_2O$ and 4.90 per cent $Al_2(SO_4)_3 \cdot 18H_2O$. These figures indicate an excessive accumulation of salts in soils receiving about 30 inches of rainfall annually.

Although the water extract of adjacent soil, the surface of which appeared to be free from deposit of salts, contained a much smaller amount of soluble salts, the quantities of aluminum and sulphate found indicate the presence of an appreciable accumulation of aluminum sulphate. The reaction of the water extract of the soil was decidedly acid, the acidity being due to the considerable quantity of aluminum sulphate present.

In another locality where a similar accumulation of salts was found at the surface, the soil was sampled in one-foot sections to a total depth of 6 feet. A qualitative examination of the samples representing the several depths showed the presence of large amounts of water-soluble sulphate and calcium, except in the sample taken to a depth of 3 feet, which gave no test for calcium in the water solution.

TABLE I
COMPOSITION OF WATER EXTRACTS OF SOILS

	Soil including salt deposit at surface	Adjacent soil
MgO	.695	.022
Al_2O_3	.753	.178
SO_3	3.845	.084
Cl	.120	trace

The sample from the surface contained considerable aluminum sulphate, but none of the lower depths showed a trace of aluminum. The water extract of the surface soil in this case was also strongly acid and that of the other depths was neutral in reaction. No water-soluble magnesium was present in this soil as compared with the first soil described, the soluble salt content of which was composed chiefly of magnesium and aluminum sulphates and contained only a small amount of calcium. No water-soluble iron was found in either case. Many iron concretions were found in soils in this vicinity.

The salts which form the main mass of the saline deposits forming the so-called alkali soils of arid regions generally include carbonates, chlorides and sulphates of sodium, potassium, calcium and magnesium. The composition of the residue on the soils described differs from these alkali soils in that the salts are either the sulphate of calcium or magnesium, together with considerable amounts of aluminum sulphate, which imparts a very strong acid reaction to the surface soil extract, as indicated by phenolphthalein and litmus paper.

The presence of aluminum sulphate can be explained as being due to the absorption of calcium or magnesium from their salts, leaving the sul-

phate radical free to combine with aluminum. Oxidation of pyrites in the soil or subsoil may be a contributing cause of the accumulation of sulphates of calcium and magnesium observed in these cases.

The lack of vegetation on the areas affected may not be due altogether to the presence of salts, although this is indicated, but to poor drainage. The soil being very impervious, it is a question whether the remedies which suggest themselves, namely: drainage and liming, will overcome the difficulty. More information as to the geological formation and a more extended study of the subsoil is necessary before the probable source of the salts can be determined.

THE YIELD AND NITROGEN CONTENT OF SOYBEANS AS AFFECTED BY INOCULATION[1]

By J. G. Lipman, *Director*, and A. W. Blair, *Associate Soil Chemist*, *New Jersey Agricultural Experiment Stations*

Soybeans lend themselves readily to comparisons of inoculating material derived from different sources. It appears that this crop is less likely to become inoculated spontaneously than other legumes which may be used in tests of the value of commercial cultures for soil inoculation. Moreover, soybeans are a satisfactory crop for the purpose just indicated, since the plants are rather hardy and may be made to grow without difficulty under a wide range of soil and climatic conditions.

The data recorded in the following pages relate to a comparison of commercial cultures as well as of soil derived from different sources. The experiments were carried out by means of 1-gallon, glazed earthenware pots. The soil employed was so treated as to make the nitrogen supply the limiting factor of growth. Two series, each containing 26 pots, were included in the experiment.

In the first of these, to be designated as Series A, there was employed a rather poor sandy loam soil possessing a distinct acid reaction. The entire amount of soil used in this series was thoroughly mixed and distributed in quantities of 10½ pounds each in the glazed earthenware pots. There were then added to the soil in each pot, and thoroughly mixed with it, 2 gm. of acid phosphate, 1 gm. of muriate of potash and 10 gm. of ground limestone. Optimum moisture conditions were established by the addition of water, and 15 seeds of the Guelph variety of soybeans were planted in each pot on June 24, 1915. Inoculation was then provided, or left out, according to the following scheme:

POTS	INOCULATION
1- 2	Check.
3- 4	Nitragin.
5- 6	Farmogerm.
7- 8	Mulford Nitrogerm.
9-10	Standard Nitrogerm.
11-12	Ferguson's Composite.
13-14	Bacto-Natural.
15-16	Soybean Soil, New Jersey Agricultural Experiment Station.
17-18	Soybean Soil, Middlesex County, New Jersey.
19-20	Cowpea Soil, Mercer County, New Jersey.
21-22	Soybean Soil, Atlantic County, New Jersey.
23-24	Soybean Soil, Sussex County, New Jersey.
25-26	Sporogen (old sample).

[1] Received for publication April 10, 1916.

Of the inoculating material named above, Nitragin was furnished by the German-American Nitragin Company, of Milwaukee, Wis.; Farmogerm, by Earp-Thomas Farmogerm Company, Bloomfield, N. J.; The Mulford Nitrogerm, by the H. K. Mulford Company, Glenolden, Pa.; the Standard Nitrogerm, by the Standard Nitrogerm Company, Glen Ridge, N. J.; Ferguson's Composite, by the Homewood Nitrogen Company, New York City; the Bacto-Natural, by Lewis Sturtevant Woodruff, of Lexington, Mass.; Sporogen, by Bruno Grosche & Co., of New York City. The last named was an old sample which had been kept in the laboratory for several years. The results secured from it should not for this reason, be accepted as a correct indication of the value of this material for inoculating purposes. It was included in the test for the purpose of determining whether positive results may be obtained from it even though it had been kept in a dry condition in the laboratory for several years.

Where inoculation was made by means of soil from different sources, an infusion was prepared in each case and equivalent quantities of such infusion were used for inoculation. The soybean plots of the Experiment Station from which a part of the inoculation material was derived have grown soybeans for several years and seem to be well supplied with bacteria capable of producing nodules on these plants. The Middlesex County soil had grown soybeans, some of which at least were known to have been inoculated. The soil from Mercer County had grown a good crop of cowpeas whose roots were well supplied with nodules. The soil from Atlantic County was claimed to have grown soybeans. There was no definite record, however, as to the facts in the case, particularly as to whether plants actually grown on the land in question had been inoculated. The soil from Sussex County had grown a good crop of soybeans whose roots were well supplied with nodules.

The seed germinated well and a good stand of plants was secured in each case. The crop was harvested on September 18, 1915, dried and weighed, and the weights recorded. The dried samples were ground and portions of the ground material were used for nitrogen determinations by the Kjeldahl method. The results secured are recorded in Table I.

On examining the data in question, we find that there is, with few exceptions, a very satisfactory agreement in the duplicates of each treatment. The check pots produced, on an average, 8.25 gm. of dry matter, whereas the inoculated soils produced, in several instances, more than twice as much dry matter. It will be observed that Nitragin, Farmogerm and the soil infusion from the Sussex County soil were particularly effective in providing for large yields. Bacto-Natural, the soil infusion from the Mercer County soil, the soil infusion from the Atlantic County soil and Sporogen did not, apparently, inoculate the soil sufficiently to

provide for an increased growth. The yield of dry matter for the soils inoculated with Ferguson's Composite was, on the average, but little greater than that from the checks.

TABLE I

RECORD OF DRY MATTER AND NITROGEN OBTAINED FROM SOYBEANS GROWN IN INOCULATION TESTS: SERIES A

No.	Inoculation	Dry Matter gm.		Inc. over check gm.	Nitrogen %	Tot. Nitrog'n mg.		Inc. over ch'k mg.
		p'rPot	Aver.			p'rPot	Aver.	
1	.....	10.5			1.299	136		
2	Check	6.0	8.25		1.358	82	109	
3	.....	22.0			3.282	722		
4	Nitragin	20.5	21.25	13.00	3.312	679	700	591
5	.....	17.5			3.371	589		
6	Farmogerm	21.0	19.25	11.00	3.331	700	645	536
7	.....	15.0			3.272	491		
8	Mulford Nitrogerm	13.0	14.00	5.75	3.412	444	468	359
9	.....	18.0			3.411	614		
10	Standard Nitrogerm	13.8	15.90	7.65	3.480	480	547	438
11	.....	7.0			2.906	203		
12	Ferguson's Composite	12.0	9.50	1.25	2.836	340	272	163
13	.....	7.2			1.884	136		
14	Bacto-Natural	6.0	6.60	.....	1.765	106	121	12
15	.....	15.0			3.212	482		
16	Soybean Soil, N. J. Agr. Exp. Sta.	22.0	18.50	10.25	3.074	676	579	470
17	.....	16.0			3.106	497		
18	Soybean Soil, Mid. Co., N. J.....	14.0	15.00	6.75	2.717	380	439	330
19	.....	7.4			1.329	98		
20	Cowpea Soil, Mer. Co., N. J.....	10.0	8.70	0.45	1.933	193	146	37
21	.....	4.5			1.805	81		
22	Soybean Soil, Atl. Co., N. J.....	7.3	5.90	.....	1.735	127	104	...
23	.....	18.0			3.312	596		
24	Soybean Soil, Sus. Co., N. J.....	26.0	22.00	13.75	3.341	869	733	624
25	.....	8.0			1.458	117		
26	Sporogen (Old Sample).....	12.0	10.00	1.75	2.627	315	216	107

The yields of dry matter gain in interest when taken in conjunction with the percentages of nitrogen in the dry matter. It will be noted that in the checks, as well as in the soils treated with Bacto-Natural and the infusions from the Mercer County and Atlantic County soils, the percentage of nitrogen in the dry matter was below 2 per cent. On the other hand, in the dry matter of the plants which had been inoculated with Nitragin, Farmogerm, Mulford Nitrogerm, Standard Nitrogerm and the infusion from the Experiment Station plots and the Sussex County soil, the percentage of nitrogen was well above 3 per cent. It is clear, therefore, that soybean plants, devoid of inoculation, not only fail to produce a large yield of dry matter when the soil is deficient in available nitrogen, but also contain a much smaller proportion of nitrogen in the plant substance than is usually found in plants that are properly inoculated. It may be pointed out, also, that there were marked differences in the effectiveness of the different cultures as well as of the different soils employed as inoculating material. Among the commercial cultures used, Nitragin

TABLE II

RECORD OF DRY MATTER AND NITROGEN OBTAINED FROM SOYBEANS GROWN IN INOCULATION TESTS: SERIES B

No.	Inoculation	Dry Matter gm.		Inc. over check gm.	Nitrogen %	Tot. Nitrog'n mg.		Inc. over ch'k mg.
		p'rPot	Aver.			p'rPot	Aver.	
1	.....	30.0			3.296	989		
2	Check	27.0	28.50	.....	3.374	911	950	...
3	.....	29.0			3.572	1037		
4	Nitragin	23.0	26.00	.....	3.582	824	931	...
5	.....	27.6			3.611	996		
6	Farmogerm	26.8	27.20	.....	3.928	1053	1025	75
7	.....	34.0			3.434	1168		
8	Mulford Nitrogerm	24.0	29.00	0.50	3.621	869	1019	69
9	.....	30.0			3.582	1075		
10	Standard Nitrogerm	28.0	29.00	0.50	3.327	931	1003	53
11	.....	31.0			3.327	1031		
12	Ferguson's Composite	31.5	31.25	2.75	3.582	1129	1080	130
13	.....	25.5			3.552	906		
14	Bacto-Natural	28.0	26.75	.....	3.395	950	928	...
15	.....	28.0			3.505	981		
16	Soybean Soil, N. J. Agr. Exp. Sta.	28.3	28.15	.....	3.464	980	981	31
17	.....	29.0			3.385	982		
18	Soybean Soil, Mid. Co., N. J.....	29.0	29.00	0.50	3.464	1005	994	44
19	.....	28.0			3.483	975		
20	Cowpea Soil, Mer. Co., N. J.....	28.0	28.00	.....	3.532	989	982	32
21	.....	28.0			3.405	953		
22	Soybean Soil, Atl. Co., N. J.....	24.3	26.15	.....	3.405	827	890	...
23	.....	32.5			3.453	1123		
24	Soybean Soil, Sus. Co., N. J.....	26.0	29.25	0.75	3.462	900	1012	62
25	.....	31.0			3.505	1086		
26	Sporogen (Old Sample).....	37.0	34.00	5.50	3.327	1230	1158	208

was evidently the most effective inoculating material; while, among the soil infusions employed, that derived from the Sussex County soil was the most effective. It may be safe to state, therefore, that commercial cultures may be fully as effective for inoculating purposes as suitable soil material, but that, under favorable conditions, soil material may prove to be fully as satisfactory as the best artificial cultures.

Another series, designated as Series B, was arranged to correspond to series A, except that the pots were filled with a silt loam soil in a good state of fertility and well provided with organisms capable of producing nodules on the roots of soybeans. The soil employed in this series had been utilized for the growing of soybeans in connection with certain plant-breeding experiments conducted by the Botanist of the Experiment Station. In this case 9 pounds of soil were placed in each pot and the optimum moisture conditions were established by the addition of water. Fifteen seeds of the Guelph variety of soybeans were planted in each pot on June 24, 1915. As in Series A, 2 gm. of acid phosphate, 1 gm. of muriate of potash and 10 gm. of ground limestone were added to and thoroughly mixed with the soil in each pot previous to the planting of the soybean seed. The crop was harvested on September 18, 1915.

The weight of the dry matter and the percentages of nitrogen in the dry matter, as found in each case, are recorded in Table II.

The yields, as recorded in this table, show that the soil employed was well supplied with the proper strain of *Bacillus radicicola.* The average yield of dry matter in the check pots was 28.50 gm. as against 8.25 gm. where soil lacking in these bacteria was employed. It appears, then, that the use of soil already inoculated would prevent the production of larger yields of dry matter where commerical cultures or soil infusions were employed. Theoretically, a further increase would have been possible only if the organisms introduced by the commercial cultures or the soil infusions were more efficient as nitrogen-fixers than those already present in the soil. A careful study of the data presented in Table II shows that the use of artificial culture material or of soil infusions did not lead to any striking increase in the yields of dry matter. Indeed, it may almost be assumed that the differences noted were within the limit of experimental error.

The same relations appear seemingly in the proportions of nitrogen present in the dry matter from the different pots. In all cases, the content of nitrogen in the dry matter was well above 3 per cent, and in several instances it was above 3½ per cent. It appears, further, that a slight, but none the less distinct, increase in the yield of total nitrogen was obtained from pots where Farmogerm, Mulford Nitrogerm, Standard Nitrogerm and Ferguson's Composite were employed. The pots in which some of the soil infusions were used also gave slight increases. The largest increase for the inoculated pots was obtained from soils which had been inoculated with an old sample of Sporogen. The average yield of dry matter from pots 25 and 26, where Sporogen was used, was 34 gm. However, this relatively high average was due largely to the yield of 37 gm. of dry matter obtained from Pot No. 26. Considering the data as they stand, there is hardly any justification for assuming that the organisms introduced by the Sporogen were responsible for the increase observed.

Summary

Taking the data in their entirety, we are led to conclude that the use of inoculating material may be very desirable in the growing of soybeans, and perhaps of other legumes. The results recorded here confirm results previously recorded by our own station or by other stations. It appears that where the soil is lacking in the right type of *Bacillus radicicola,* inoculation is eminently desirable, and that, even where the organisms are present in limited numbers, the addition of larger numbers may be profitable. It appears, further, that there is a marked difference in quality of different commercial preparations for soil inoculation and that soils de-

rived from different sources may vary as widely as, though not more widely than, commercial cultures as to their effectiveness in promoting nitrogen fixation by legumes.

The authors take this opportunity of expressing appreciation for assistance rendered by Mr. H. C. McLean and Mr. L. K. Wilkins of the Department of Soils of this station.

STUDIES ON SOIL COLLOIDS

I. FLOCCULATION OF SOIL COLLOIDAL SOLUTIONS[1]

By M. I. Wolkoff, *Michigan Agricultural College*

Introduction

The present status of our knowledge of flocculation of soil particles is based largely upon three sources of information, namely: (a) deductions from general colloidal chemistry, (b) studies with kaolin, and (c) studies with different clays.

From theoretical considerations the deductions from facts established with pure colloidal solutions are very valuable and should serve as guides in further investigations with such a complex medium, the soil.

Selmi (38) and Graham (16) have observed that salts and acids added to colloidal solutions cause its coagulation. Further, it was noticed that only electrolytes bring about coagulation, while non-electrolytes do not (5) possess this property at all, or only to a very small degree (30) when present in concentrated solutions. Later it was observed that some colloids, if placed in an electric field, move toward the cathode, while others gather themselves around the anode. According to this action they are classified as positive and negative colloids, respectively. Hardy (18) established the fact that the iron which coagulates a given colloid moves toward the opposite pole from the one to which the colloid moves. Linder and Picton (25) found that coagulation of colloids with negative electric charges is accompanied by the absorption of the positively charged ions of the electrolyte.

Besides the electrolytes and the familiar action of heat and frost (33), as discussed by Ostwald, there are other agencies which influence the stability of colloidal solutions. Several cases on record (40, 41, 14) showing that, when two different colloidal solutions with opposite electric charges are mixed together, the coagulation takes place. Radium rays help considerably in the coagulation of colloidal $Fe(OH)_3$ (23) by minute quantities of electrolytes which, if acting alone, are too dilute to cause a coagulation. Later it was found (31) that without the aid of an electrolyte, light from different sources acts as a slow coagulant, resembling in its behavior a weak electrolyte. Von Veimann and Alekseyev (42) have demonstrated that several, both positively and negatively charged, colloids can be coagulated at will by merely shaking a given colloidal solution for a sufficient length of time with the insoluble liquids or solids.

[1] Received for publication May 3, 1916.

[2] The experimental results are taken from the author's thesis presented to the faculty of Michigan Agricultural College as a partial fulfillment of the requirement for the degree of M.Sc.

The foregoing are a few of the facts from colloidal chemistry which are helpful guides in understanding the phenomenon of flocculation in the soil colloidal solutions. The direct investigations in such solutions, however, are of more value for the investigator of soils because of the fact that the properties of even pure colloidal solutions vary greatly. Certainly, the variations increase enormously when one deals not with a solution of a single colloid but with a mixture of several colloids, in addition containing numerous salts in the true solution. In such a case the resultant of all these factors must be taken into consideration.

In order to throw light upon the properties of soil colloids a number of workers studied suspensions of kaolin, while others studied different clay suspensions either alone or in parallel with kaolin and other suspensions.

As early as 1866, or only a few years after the publication of Graham's classical investigations on colloidal substances, Schulze (36) recorded some of his results on the calcium and magnesium salt requirements for flocculation of clay suspensions. Later Schloessing (35) worked along the same line. Durham (12, 13)made an interesting discovery that although it requires a very small amount of sulphuric acid to flocculate the suspension of white clay (kaolin?), on further additions of sulphuric acid he reached the point when suspension did not clarify for a long time. Now, if to this mixture of clay suspension and sulphuric acid he added either more acid or some water, the suspension clarified quickly. Evidently, there is an equilibrium between the ions of true solution and the solid particles of clay. The flocculating action of sodium carbonate, on the other hand, continued to increase with the increase in concentration.

While working on the method of mechanical analysis, Hilgard (20, 21) noticed that clay suspension coagulated on passing through the narrow glass tube and flocculation is approximately inversely proportional to the size of the particles. A moderate increase in temperature decreased the flocculation in his case. He also studied the effect of lime on the texture of clays (19). Brewer (10) found the different clay suspension to be of different stability. In fact, some suspensions settle within a few days, while others remain turbid at the end of seven years, when kept at nearly the same temperature and in a quiet place. The acids he found to flocculate more quickly than the salts. Barus (5) in 1888 observed that non-electrolytes retard the clearing of suspensions. Later (6) he tested the hypothesis that the hydration of clay or kaolin particles is responsible for keeping their particles in suspention and came to the conclusion that such is not the case. He determined the densities of tripoli and bole in both water and ether and found them to be the same in both liquids. Since tripoli has practically the same density as quartz,

and bole approaches that of kaolin, he justified his conclusion on these grounds. Spring (39) noticed that the clearing power of salt depends upon the valence of the salt and the cation of the electrolyte, confirming in part the quantitative formula of Schulze (37) that the coagulating power of trivalent cation: divalent: monovalent as 350:20:1.

Bodländer (9) also measured the power of different salts for clearing the kaolin suspensions. Quincke (34) from his studies on pure colloids and kaolin suspensions advanced a theory on coagulation which in short implies the change in surface tension between the liquid and the oily substances. He claims to have observed oily films around the solid particles. Hall and Morison (17) while studying the efficiency of electroytes in flocculating the kaolin suspensions found that the order of efficiency of acids to be $HCl > HNO_3 > H_2SO_4$. In the case of cations of salts it is $Al > Ca > K > Na$. Acids are better coagulants than their salts. Exceptions are $Al_2(SO_4)_3$ which is equal to H_2SO_4, but does not exceed it. Maschhaupt (29) found that NaOH stabilizes soil suspensions at low concentrations, while, if present above .015 N, it causes flocculation. Similar results were obtained with Na_2CO_3 in which case the coagulation begins above 0.16 N. Oden (32) in rather extensive studies with peat colloidal solutions used NaCl for the flocculation. He had to saturate his colloidal solution with the pure salt and allow it to stand for 24 hours in order to bring about flocculation. McGeorge (28) working with suspensions of Hawaiian clays obtained results similar to those of Hall and Morison with the exceptions that he found $Al_2(SO_4)_3$ to be the best flocculant among both salts and acids and the order of efficiency of strong acids was $HNO_3 > HCl > H_2SO_4$.

This short review of the past investigations on coagulation or flocculation, the term generally used in soil investigations, does not claim completeness. It reveals, however, a striking fact that practically all the work along this line has been done either with clays or kaolin. The later substance, which is very unplastic, crystalline in nature and remains in suspension only for a short time, can hardly be classified with the colloidal substances. No record of any importance was found in the literature bearing on the attempts to study suspensions of other soils besides clays. Yet it is a well known fact that no two colloidal solutions possess exactly the same properties toward the action of an electrolyte, and this is much more striking in the case of soil colloidal solutions, for undoubtedly one deals not with a single colloidal solution but with a mixture of several of them. The relation of one colloidal substance to another in such a mixture must be different with different soils, depending on the origin of the soil, its chemical composition, age, climate, etc. For this reason it was considered of sufficient importance to study the behavior of different classes of soils with respect to different electrolytes in order to better understand the phenomenon of flocculation in the soil.

EXPERIMENTAL

Method. The soil colloidal solutions were prepared by adding to a bulk of fresh soil about 10 times its weight of distilled water, shaken well and allowed to stand over night. Then, the supernatant liquid was siphoned off and centrifuged at the rate of 2000 revolutions a minute for 15 minutes. The resultant solution would stand for several weeks and even months without appreciable sedimentations. In most of the experiments here recorded the same solutions were used. The exceptions will be mentioned later.

NATURE OF SUSPENSIONS USED

N. Soil Used	Reaction of soil with litmus paper	Dry Matter per 100 c.c. of suspension	Freezing point depression of solution
1. Brickyard clay (subsoil)..	neutral	.3633	.003
2. Miami silt loam	neutral	.0700	.002
3. Clyde silt loam	neutral	.0913	.003
4. Muck	neutral	.0274	.002
5. Brickyard clay (soil)	neutral	.8098	....
6. Peaty muck	neutral	.0338	....
7. Kaolin	.....	.0247	....

The bacterial action in the colloidal solutions during the experiment was not controlled.

The acid, salt and alkali solutions were N/5 in strength and were the same throughout the experiments.

Experiment I. Qualitative test of electrolytes for flocculation of colloidal solutions.

In this experiment to 5 c.c. of suspension was added 5 c.c. of N/5 electrolyte, shaken vigorously for a short time and allowed to stand over night. Five positive signs ***** were recorded for the solution which

TABLE I

EFFICIENCY OF ELECTROLYTES IN FLOCCULATING SOIL COLLOIDAL SOLUTIONS

5 c.c. of Electrolyte N/5	1 Clay (subsoil)	2 Miami Silt Loam	3 Clyde Silt Loam	4 Muck	5 Clay (soil)	6 Peaty Muck	7 Kaolin
1. HCl	*****	*****	*****	*****	*****	*****	*****
2. NaCl	*****	—	—	—	*****	—	*****
3. KCl	*****	****	****	—	*****	—	****
4. NH_4Cl	*****	***	***	—	*****	—	*****
5. $BaCl_2$	*****	*****	*****	*****	*****	*****	*****
6. $CaCl_2$	*****	*****	*****	****	*****	****	*****
7. $HgCl_2$	*****	—	—	—	****	—	—
8. $MgCl_2$	*****	*****	*****	*	*****	*	*****
9. $SnCl_4$	*****	***	***	****	*****	****	*****
10. HNO_3	*****	*****	*****	*****	*****	*****	*****
11. $NaNO_3$	*****	—	—	—	*****	—	*****
12. KNO_3	****	***	***	—	****	—	*****
13. NH_4NO_3	*****	***	***	—	*****	—	*****

TABLE I—(Continued)

EFFICIENCY OF ELECTROLYTES IN FLOCCULATING SOIL COLLOIDAL SOLUTIONS

5 c.c. of Electrolyte N/5	1 Clay (sub-soil)	2 Miami Silt Loam	3 Clyde Silt Loam	4 Muck	5 Clay (soil)	6 Peaty Muck	7 Kaolin
14. $Ca(NO_3)_2$	*****	*****	*****	*****	*****	*****	*****
15. $Hg_2(NO_3)_2$	*****	*****	*****	*****	*****	*****	*****
16. $AgNo_3$	*****	*****	*****	*****	*****	*****	*****
17. $Pb(NO_3)_2$	*****	*****	*****	*****	*****	*****	*****
18. H_2SO_4	*****	*****	*****	*****	*****	*****	*****
19. $KHSO_4$	*****	*****	****	***	*****	***	*****
20. $(NH_4)_2SO_4$	****	—	—	—	*****	—	*****
21. K_2SO_4	*****	*****	***	—	*****	—	*****
22. $K_2S_2O_7$	*****	*****	*****	***	*****	***	*****
23. $MnSO_4$	*****	*****	****	**	*****	**	*****
24. $CuSO_4$	*****	****	*****	***	*****	***	*****
25. $FeSO_4$	*****	*****	*****	****	*****	****	*****
26. $Fe_2(SO_4)_3$	*****	*****	*****	*****	*****	*****	*****
27. K_2S	****	***	***	—	*****	—	—
28. $NaSO_3$	****	—	—	—	*****	—	*
29. $NaHSO_3$	****	—	—	—	*****	—	—
30. $Na_2S_2O_3$	****	—	—	—	****	—	—
31. $AlK(SO_4)_2$	*****	*****	*****	*****	*****	*****	*****
32. $Fe(NH_4)_2(SO_4)_2$	*****	*****	*****	*****	*****	*****	*****
33. FeS	—	—	—	—	—	—	*****
34. ZnS	—	—	—	—	—	—	—
35. NaOH	*****	****	***	*	*****	—	*****
36. KOH	*****	*****	*****	—	*****	—	*****
37. $Ba(OH)_2$	*****	*****	*****	*****	*****	*****	*****
38. MgO	****	—	—	—	*****	—	****
39. CaO	*****	*****	*****	*****	*****	*****	*****
40. H_3PO_4	*****	*****	*****	*****	*****	*****	*****
41. $NaHPO_4$	*****	—	—	—	*****	—	***
42. $CaH_4(PO_4)_2$	**	—	—	—	*	—	—
43. $Ca_3(PO_4)_2$	—	—	—	—	—	—	—
44. KH_2PO_4	****	*	*	—	*****	—	—
45. K_2HPO_4	****	*	**	—	*****	—	—
46. K_2CO_3	*****	***	***	—	*****	—	—
47. Na_2CO_3	***	—	—	—	****	—	—
48. $NaHCO_3$	****	—	—	—	****	—	—
49. $CaCO_3$	—	—	—	—	—	—	—
50. $3MgCO_3Mg(OH)_2$	****	—	—	—	*****	—	***
51. $FeCO_3$	—	—	—	—	—	—	—
52. $(NH_4)_2CO_3$	****	—	—	—	*****	—	—
53. CH_3COOH	*****	****	***	—	*****	—	—
54. $NaC_2H_3O_2$	*****	—	—	—	*****	—	*****
55. $Ca(C_2H_3O_2)_2$	*****	*****	*****	****	*****	****	*****
56. $Pb(C_2H_3O_2)_2$	*****	*****	*****	*****	*****	*****	*****
57. $C_2H_2O_4$	*****	***	****	****	*****	****	*****
58. $C_6H_8O_7$	*****	*****	*****	*****	*****	*****	*****
59. $C_{17}H_{35}COOH$	—	—	—	—	—	—	—
60. As_2O_3	—	—	—	—	—	—	—
61. $(NH_4)_2C_2O_4$	*****	*****	*****	—	*****	—	*
62. $NaKC_4H_4O_6$	*****	—	***	—	*****	—	*****
63. KBr	*****	*****	*****	—	*****	—	*****
64. KI	*****	*****	*****	—	*****	—	*****
65. KSCN	*****	*****	*****	—	*****	—	*****
66. PbO_2	—	—	—	—	—	—	—
67. $K_2Cr_2O_7$	*****	***	***	*	*****	—	****

was floculated the most. The one next in apparent efficiency was marked ****, and so on until a negative sign was used if no precipitate appears at the bottom of the test tube in 24 hours. Duplicate determinations were made in all of the experiments.

The results presented in Table I show that besides the familiar difference in efficiencies of different electrolytes with the same colloidal solution, the same electrolyte does not act alike with different suspensions, the easiest solutions to flocculate being that of clay and kaolin, followed by loams and, finally, mucks. This question is almost entirely overlooked by many soil investigators. As it was pointed out in the introductory remarks of this article, no studies have been recorded in soil literature, so far as the writer has been able to determine, on the flocculation of suspensions other than those of clay and kaolin. As a result, the conclusions regarding this process (perhaps as well as others) in soils have been based upon the results obtained from studies with a limited number of soils. But such conclusions, judging from the results presented in Table I may be erroneous, due undoubtedly to the fact that soils differ one from another in many respects, namely: chemically, physically and biologically. They may have different origin and different history with respect to their management. When taken from the same locality, as they were in this case, they may have only one factor in common, namely—climate. Very probably, a given type of soil, if exposed to different climatic conditions for a sufficient length of time, would behave differently with the same electrolyte. For instance, Lipman and Waynick (26) in a recently published article showed that the colloidal content of a Kansas soil, as judged from the suspended material after standing for 24 hours, was considerably modified by placing it in the climate either of California or of Maryland.

Strong acids are very good coagulants but they are not always better than some of their salts. This point is especially well brought forth by the next experiment. The salts of the heavy metals used have a much stronger flocculating power than those of lighter ones with respect to these soils. The trivalent cation is more efficient than a divalent one and this latter is better than a monovalent cation. Yet the tetravalent stannic chloride does not seem to do as efficient work as the divalents, barium chloride or calcium chloride. Contrary to the prevalent opinion, bases flocculate when used in this concentration. Only muck resists monovalent bases and yields fairly easily to divalents, both barium hydroxide and calcium hydroxide.

Experiment II. The minimum amount of electrolyte in solution required for the flocculation of a given amount of soil colloidal solution.

For this experiment all colloidal solutions were brought to as nearly the same concentration, as was possible. All stock colloidal solutions were so diluted that they contained .02735 gm. of dry material when 100

c.c. of solution was evaporated. To determine the minimum electrolyte requirement the following procedure was adopted.

Ten c.c. of colloidal solution was placed in each of a series of from 8 to 16 tubes, 25 c.c. graduated tubes being used as containers. Then, to the tube No. 1 was added 0.1 c.c. of N/5 salt solution; to No. 2, 0.2 c.c.; to No. 3, 0.3 c.c., etc. increasing gradually the amount of salt added. The solutions were vigorously shaken and allowed to stand over night. Now, if solutions in tubes Nos. 1, 2, and 3 have not settled while the rest of the solutions clarified, then .4 c.c. of that salt or acid was the requirement recorded. Often all solutions in a series was prepared with 15, 20, 25 or even 50 c.c. to which the small quantities of a flocculant were added. The recorded results, however, for convenience were all calculated to indicate the requirement per 10 c.c. of colloidal solution.

TABLE II

MINIMUM ELECTROLYTE REQUIREMENT FOR COAGULATION OF 10 C.C. OF SOIL COLLOIDAL SOLUTIONS OF EQUAL CONCENTRATIONS

Electrolyte N/5	1 Clay (subsoil) c.c.	2 Miami Silt Loam c.c.	3 Clyde Silt Loam c.c.	4 Muck c.c.	5 Clay (soil) c.c.	6 Peaty Muck c.c.
HCl	.033	.100	.10	.200	.033	.20
$BaCl_2$	.050	.100	.15	.300	.050	.40
$CaCl_2$	.100	.200	.20	1.000	.100	1.50
$MgCl_2$	.100	.500	.50	Negative with 10 c.c.	.100	Negative with 10 c.c.
$SnCl_4$	.050	.100	.10	.200	.050	.15
HNO_3	.050	.100	.10	.200	.050	2.0–3.0
$Ca(NO_3)_2$	.100	.200	.20	2.000	.05–.1	2.0–3.0
$Hg_2(NO_3)_2$	.033	.033	.05	.050	.020	.10
$AgNO_3$	.500	1.000	1.00	1.000	.300	...
$Pb(NO_3)_2$	.020	.033	.05	.033	.020	.10
H_2SO_4	.050	.100	.15	.200	.050	.25
$KHSO_4$	.100	.150	.20	.300	.100	.40
$K_2S_2O_7$	.100	.150	.30	.500	....	.60
$MnSO_4$	.033	.150	.15	1.500	....	...
$CuSO_4$	.025	.050	.10	.100	....	...
$FeSO_4$	.025	.150	.10	.300	....	...
$Fe_2(SO_4)_3$	.025	.025	.05	.100	....	...
$AlK(SO_4)_2$	.020	.020	.05	.100	....	...
$Fe(NH_4)_2(SO_4)_2$.	0.25	.200	.15	1.500	....	...
NaOH	1.500	5.000	Negative with 10 c.c.	Negative with 12 c.c.	....	...
$Ba(OH)_2$	.100	.150	.20	2.000	....	...
$Ca(OH)_2$	.200	.300	1.00	2.000	....	...
H_3PO_4	.100	.200	.30	1.000	....	...
$Ca(C_2H_3O_2)_2$ ...	.050	.150	.15	2.000	....	...
$Pb(C_2H_3O_2)_2$ ...	.020	.025	.05	.035	....	...
$C_7H_2O_4$	2.000	....	...	.500	....	...
$C_6H_8O_7$	.150	1.500	2.00	1.000	....	...
$(NH_4)_2CO_3$	3.000		Negative	with 10 c.c.		

The results presented in Table II and figure 1 show the difference in the efficiency of different electrolytes with different soils much better than the qualitative results in the Table I. The trivalent ferric sulphate and

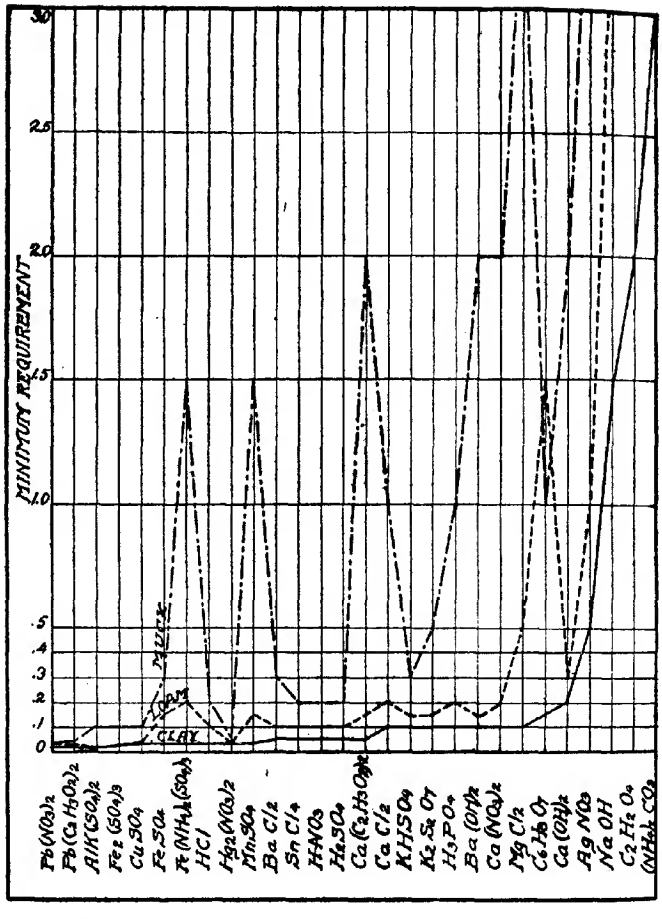


Fig. 1.—The minimum electrolyte requirement for coagulation of soil colloidal solutions.

aluminum potassium sulphate are not the leading ones; the salts of lead, being only divalent, both nitrate and acetate act better, especially in the case with muck solutions. There is not the slightest indication of following the formula of Schulze (37). As the chart shows, the silt is more resistant to the action of electrolytes than clay, and muck is the most resistant of the three selected classes. There is one striking fact brought out by this chart. With the best coagulants the minimum electrolyte re-

quirement of all solutions is nearly the same, as one notices in the cases of $Pb(NO_3)_2$, $Pb(C_2H_3O_2)_2$, $Hg_2(NO_3)_2$ and to some extent $Fe_2(SO_4)_3$ and $AlK(SO_4)_2$, but with others the variations are great and often very irregular, evidently being dependent not only upon the cation but also upon the anion, the chemical composition of the colloidal particles and the salts present in the original solutions. Undoubtedly, an important rôle is played by the so-called humic substances of the soil whose protective action was suggested by Fickendey (15) and later by Keppeler and Spangenberg (24); and perhaps the similar observations mislead Lyon, Fippin and Buckman (27) to make the statement that "the gelatinous colloids of the soil, such as some of the humic materials, are not agglutinated by the addition of electrolytes."

In order to ascertain to what extent this difference in resistance of colloidal solution to the flocculating action of electrolyte could be ascribed to the protective influence of humic material, an experiment was undertaken and the following obtained results may typify the case.

Experiment III. Effect of muck colloidal solution on the stability of clay colloidal solution.

The clay colloidal solution was mixed with muck colloidal solution in proportions from 100 per cent to 0 per cent of clay. The minimum electrolyte requirement of these resultant solutions was determined in the usual way. Both clay and muck suspensions were freshly prepared and the dry matter in both of them, as well as in the mixtures, was determined.

TABLE III

EFFECT OF ORGANIC MATTER ON THE MINIMUM ELECTROLYTE REQUIREMENTS FOR COAGULATION OF CLAY COLLOIDAL SOLUTION

Clay Solution	Muck Solution	Dry weight per 100 c.c. of sol.	Electrolyte Requirement	
			$Ca(OH)_2$ satur. at 20° C. per 10 c.c. of sol.	HNO_3 n/5 per 10 c.c. of sol.
100	0	.0730	.3 c.c.	.05 c.c.
75	25	.0578	.4 c.c.	.10 c.c.
50	50	.0411	.6 c.c.	.15 c.c.
25	75	.0261	1.4 c.c.	.20 c.c.
0	100	.0105	2.8 c.c.	.25 c.c.

The figures in Table III leave no doubt regarding the influence of organic material upon the stability of the solution. That the difference in stability of the colloidal solutions in the foregoing experiment was not due to the difference in their solid material content, but rather regardless of it, is absolutely proved by the next experiment.

Experiment IV. Effect of solid material present on the stability of soil colloidal solution.

The original stock solution of clay from Experiment I, was diluted 2, 8, and 32 times and the minimum coagulant requirement of each solution was determined.

TABLE IV

EFFECT OF THE CONCENTRATION OF CLAY COLLOIDAL SOLUTION ON THE MINIMUM ELECTROLYTE REQUIREMENT

Concentration per 100 c.c. of solution. Relation	Gm.	$CaCl_2$ n/25 c.c.	$Ca(NO_3)_2$ n/25	$Ca(OH)_2$ saturated	$CaSO_4$ saturated	H_2SO_4 n/25	$AlK(SO_4)_2$ n/25	$KHSO_4$ n/5	K_2SO_4 n/5	$FeSO_4$ n/25	HNO_3 n/25
1	.18165	.3–.4	.3–.4	.3–.4	.3–.4	.5	.4	.2	.9	.4	.4
¼	.04541	.3	.3	.2	.3	.3	.2	.1	.6	.15	.2–.3
1/16	.01135	.2	.2	.2	.2	.3	..	.1	.4	.1	.2

Muck colloidal solution was freshly prepared, a portion of which was diluted to 1/3 of its original concentration, and the electrolyte requirement per 10 c.c of each solution follows:

TABLE IV—A

EFFECT OF THE CONCENTRATION OF MUCK COLLOIDAL SOLUTION ON THE MINIMUM ELECTROLYTE REQUIREMENT

Electrolyte n/5	Original	1/3 of Original
$AlK(SO_4)_2$	0.10 c.c.	0.050 c.c.
$Fe_2(SO_4)_3$	0.10 c.c.	0.050 c.c.
$Pb(NO_3)_2$	0.05 c.c.	0.038 c.c.

The results indicate very plainly, first, that with the decrease of concentration of colloidal solution the minimum electrolyte requirement for flocculation of that solution decreases also. For the solutions used this is true without exception. Second, the decrease in the minimum coagulant requirement is not proportional to the decrease in concentration of colloidal solution. This lack of proportionality is probably due to the mechanical difficulties of bringing the particles together to form an aggregate large enough for stopping the Brownian movement, since there are less chances for particles to strike a certain number of particles in a dilute solution than in a concentrated one.

There is a great deal of speculation regarding the nature of coagulation. Some authors describe it as a purely physical phenomenon, while others seem to favor the application of chemical laws to the same effect observed. A few examples will illustrate the point.

Whitney (43) explains the flocculation by means of surface tension. Using his own words: "If the potential of the surface particle of water is less than of a particle in the interior of the mass of liquid there will be surface tension and the two grains will not come together, because they would enlarge the surface area and increase the number of surface particles of the liquid. If, on the other hand, the potential of the particle on the surface of the liquid is greater than the potential of a particle in the interior of the liquid mass, the surface will tend to enlarge and the grains

of clay may come close together and be held there with some force, as their close contact increases the number of surface particles in the liquid around them. This probably explains the phenomenon of flocculation."

Quincke (34) later proposed a similar theory employing the change in surface tension between liquid and the oily substances, around the solid particles. Bary (7) thought that liquid penetrates the solid particles and the attraction between the two balances itself against the elasticity of the solid and the surface tension. Upon the addition of an electrolyte the osmotic pressure is changed, causing the withdrawal of water from the colloidal particles and coagulation results. Bancroft (3, 4) in his recently published articles, summarizing the most important investigations on the subject, comes to the conclusion that in coagulation the adsorption is taking place only at the surfaces of the solid particles.

Duclaux (11), on the other hand, considers the colloids as electrolytes with the power of ionization and, although, the stability of colloidal solution is based on the equilibrium between the intermicellar liquid and the colloidal particles proper, yet the disturbance of this equilibrium implies the chemical change. Jordis (22) also attributes the coagulation to the chemical action. The similar view is held by Ashley (2). Arrhenius (1) noticed a close analogy between agglutinization and the precipitation and concluded their nature to be the same, i. e. the chemical. The recent work of Beam and Eastlack (8) on the electrical synthesis of colloids shows that in the preparation of the hydrosols there is a very close association between the colloidal particles and the ions of some electrolyte, which give the stability to that hydrosol. In order to destroy the stability, or to bring about a coagulation, there is necessary more than a mere physical change.

The following experiment, which suggested itself by an accident, seems to throw some light upon the phenomenon of flocculation.

Experiment V. Effect of concentration of colloidal solution on the time required for coagulation, the amount of electrolyte added remaining the same.

In this experiment the clay and muck colloidal solutions were diluted to ½, ¼, ⅛, etc., of their original concentrations. To 5 c.c. of each of the resultant solutions was added 5 c.c. of electrolyte N/5, vigorously shaken for a few seconds and set aside. The time in minutes when the first floccules could be observed was recorded in Table V.

The results reveal a striking regularity of time requirement by differently diluted colloidal solutions. With the exceptions of the most concentrated solutions and the minor discrepancies in a few cases, the time necessary for flocculation is nearly inversely proportional to the concentration of that colloidal solution, or it is a splendid demonstration of the mass action law stating that "the velocity of a chemical reaction is pro-

portional to the quantities present in condition to react." In our case the amount of electrolyte added was the same in all cases and always present in abundance, while another component, the colloid solution, varied, and, being a limiting factor, altered the velocity of reaction.

TABLE V

EFFECT OF THE CONCENTRATION OF COLLOIDAL SOLUTION ON THE TIME REQUIRED FOR COAGULATION

CLAY COLLOIDAL SOLUTION

Concentration of colloid solution per 100 c.c.		.3633		.18165		.090825		.0454		.0227		.01135	
Electrolyte used N/5	Temp. Degrees C.	Observed min.	Calculated	Observed min.	Calculated	Observed min.	Calculated	Observed min.	Calculated	Observed min.	Calculated	Observed min.	Calculated
HCl	21.9	0.5	3.50	4.5	.70	14.0	14.0	31.0	27.5	66.0	55.0	109	109
H_2SO_4	25.0	0.5	1.90	3.5	3.75	9.0	7.5	17.0	15.0	32.0	30.0	60	60
HNO_3	21.0	0.5	5.60	4.0	11.20	11.0	12.5	28.0	25.0	55.0	50.0	100	100
H_3PO_4	21.6	1.0	3.75	6.0	7.50	13.0	15.0	30.0	30.0	64.0	60.0	120	120
CH_3COOH	22.0	1.0	3.50	7.0	7.00	17.0	14.0	30.0	27.5	60.0	55.0	110	110
$Cr_2H_2O_4$	21.0	2.5	8.80	7.0	17.50	26.0	35.0	52.0	70.0	140.0	140.0	...	...
$C_6H_8O_7$	21.0	0.5	3.10	1.5	6.30	11.0	12.5	21.0	25.0	50.0	50.0	100	100
$CaCl_2$	23.7	0.5	2.00	4.0	4.00	11.0	8.0	26.0	16.0	38.0	32.0	64	64
$Ca(NO_3)_2$	19.2	1.0	2.80	5.0	5.60	13.0	11.1	29.0	22.2	45.0	44.5	89	89
$FeSO_4$	20.8	3.0	3.12	6.5	6.25	13.0	12.5	24.0	25.0	54.0	50.0	100	100
KOH	20.8	3.0	4.00	9.0	8.00	21.0	16.1	39.0	32.2	57.0	64.5	129	129

TABLE V—A

MUCK COLLOIDAL SOLUTION

		Original		½ Original		¼ Original		⅛ Original	
$AlK(SO_4)_2$	20.2	8	13	24	26	53	51.5	103	103
$Fe_2(SO_4)_3$	21.0	3	11.–	11	21.5	23	43	86	86
$Pb(NO_3)_2$	21.4	1½	2½	4	5	10	10.5	21	21

The figures on the right side of the column are calculated, taking the result of the most dilute solution for a basis. The close agreement between the results observed and the theoretical values is still better demonstrated by figures 2 and 3.

Taking into consideration the fact that the reactions were allowed to take place at room temperature, which necessarily fluctuated in the course of time needed for the completeness of experiment with each electrolyte studied, one notices the close coincidence of the two lines, which seem to indicate that there is a close relation between the chemical reactions and the reaction between the electrolyte and the colloidal particles or, rather, the ions associated with those particles. However, whether a flocculation is a chemical reaction, or a reaction that only obeys the chemical law is more than we can say from the results thus far at our disposal.

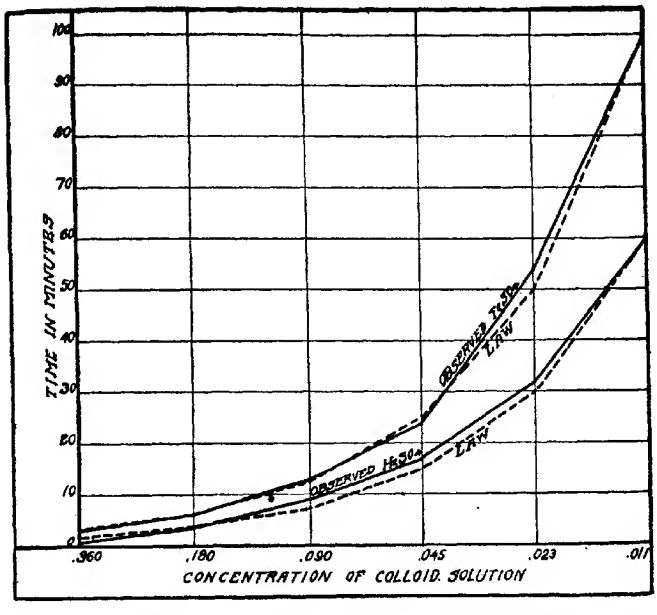


Fig. 2.—The relation between the Mass Action Law and the flocculation of clay colloidal solution.

SUMMARY

1. Besides the fact that the flocculating efficiency of different electrolytes with the same colloidal solution is different, the results show that the efficiency of the same elcctrolyte with the solutions from different soils varies considerably, depending largely upon the chemical composition of the soils.

2. Schulze's valency law does not hold true with the soil colloidal solutions studied.

3. Humic materials hinder the coagulating power of the electrolytes.

4. It takes a greater amount of electrolyte for flocculation of a more concentrated soil colloidal solution than that for a less concentrated one.

5. In the flocculation of the soil colloidal solutions by the electrolyte, the reaction obeys, within the experimental error, the law of mass action.

The author wishes to acknowledge his sincere gratitude to Dr. M. M. McCool, Professor of Soils, for his many valuable suggestions during the work as well as for critically reading the manuscript.

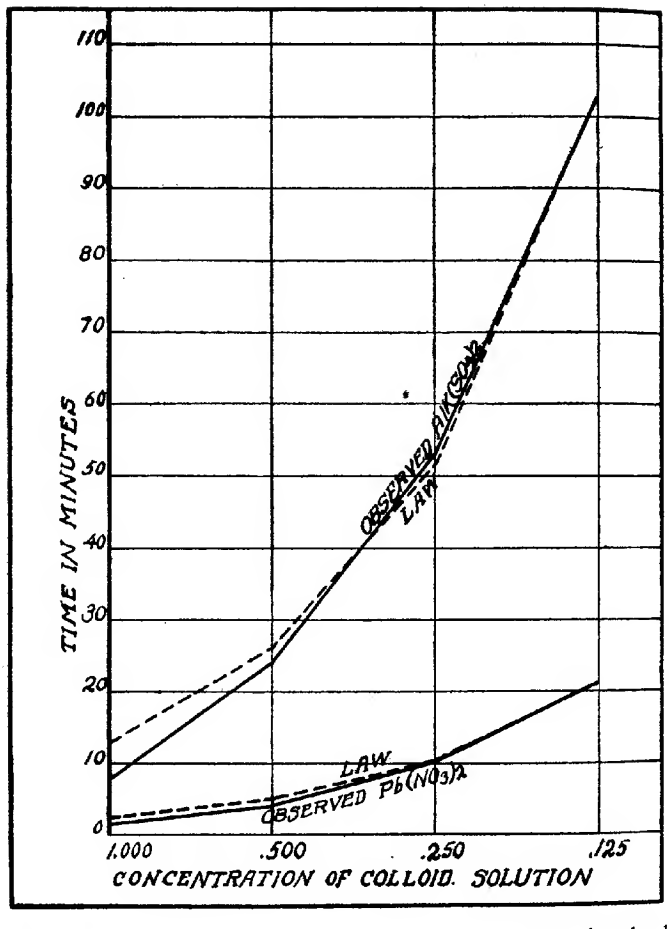


Fig. 3.—The relation between the Mass Action Law and flocculation of muck colloidal solution.

LITERATURE CITED

(1) ARRHENIUS, S.
1908. On agglutination and coagulation. *In* Jour. Amer. Chem. Soc., v. 30, p. 1382.

(2) ASHLEY, H. E.
1909. The colloid matter of clay and its measurement. U. S. Geol. Survey Bul. 388, p. 15.

(3) BANCROFT, W. D.
1915. Coagulation of albumen by electrolytes. *In* Jour. Phys. Chem., v. 19, p. 349-360.

(4) BANCROFT, W. D.
1915. Neutralization of adsorbed ions. *In* Jour. Phys. Chem., v. 19, p. 366.

(5) BARUS, C.
1888. Ueber das Setzen von feinen festen Massentheilchen in Flüssigkeiten. *In* Beibl. Ann. Phys. u. Chem., Bd. 12, p. 563-565. Cited by Quincke (see Reference No. 34). A review of U. S. Geol. Survey Bul. 36.

(6) BARUS, C.
1889. The subsidence of fine solid particles in liquids. *In* Amer. Jour. Sci., v. 37, 3 s., p. 122.

(7) BARY, P.
1911. The manner of solution of colloidal substances. *In* Compt. Rend. Acad. Sci. [Paris], t. 152, p. 1386-1387; Chem. Abs., v. 5. (1911), p. 2770.

(8) BEANS, H. T., and EASTLACK, H. E.
1915. The electrical synthesis of colloids. *In* Jour. Amer. Chem. Soc., v. 37, p. 2667-2683.

(9) BODLÄNDER, G.
1893. Versuche über Suspensionen. I. *In* Nachr. Königl. Gesell. Wiss. Georg-Augusts-Univ. Göttingen. 1893. p. 267-276. Cited by Quincke (see Reference No. 34).

(10) BREWER, W. H.
1885. On the suspension and sedimentation of clays. *In* Amer. Jour. Sci., v. 29, 3 s., p. 1.

(11) DUCLAUX, J.
1911. Colloids considered as electrolytes. *In* Ztschr. Chem. Indus. Kolloide, v. 7, p. 73-81; Chem. Abs., v. 5 (1911), p. 1226.

(12) DURHAM, W.
1874. Suspension of clay in water. *In* Chem. News, v. 30, p. 57.

(13) DURHAM, W.
1878. Suspension, solution, and chemical combination. *In* Chem. News, v. 37, p. 47.

(14) ELLIS, R.
1914. Properties of oil emulsions. *In* Ztschr. Phys. Chem., Bd. 89, p. 145-50; Chem. Abs., v. 6 (1915), p. 1559.

(15) FICKENDEY, E., and TOLLENS, B.
1906. A note on the protective action of colloids on suspended clay and natural clay soils. *In* Jour. Landw., Bd. 54, p. 343. Cited by Keppler and Spangenberg (see reference No. 24).

(16) GRAHAM, T.
1861. Liquid diffusion applied to osmosis. *In* Phil. Trans. Roy. Soc. London, v. 151, p. 183-224.

(17) HALL, A. D., and MORISON, C. G. T.
1907. The flocculation of turbid liquids by salts. *In* Rothamsted Mem. Agr. Sci., v. 8, p. 244.

(18) HARDY, W. B.
1900. A preliminary investigation of the conditions which determine the stability of irreversible hydrosols. *In* Proc. Roy. Soc. London, v. 66, p. 110-125.

(19) HILGARD, E. W.
1877. Cited by Johnson, S. W., on the mechanical effects of tillage. *In* 11th Ann. Rpt. Conn. Bd. Agr. (1877), p. 136.

(20) HILGARD, E. W.
1879. On the flocculation of particles, and its physical and technical bearings. *In* Amer. Jour. Sci., v. 17, 3 s., p. 205.

(21) HILGARD, E. W.
1879. Ueber die Flackung kleiner Theilchen und die physikalischen und technischen Beziehungen dieser Erscheinung. *In* Forsch. Geb. Agr. Phys., Bd. 2, p. 441-455. First published in Amer. Jour. Sci. Arts, s. 3, v. 17, p. 205-214, entitled "On the flocculation of particles, and its physical and technical bearings."

(22) JORDIS, E.
1908. Silicates and the chemistry of colloids. *In* Ztschr. Electrochem., Bd. 13, p. 525; Chem. Abs., v. 2 (1908), p. 1225.

(23) JORISSEN, W. P., and WOUSTRA, H. W.
1911. The action of radium rays on colloids. *In* Ztschr. Chem. Indus. Kolloide, Bd. 8, p. 8-11; Chem. Abs., v. 5 (1911), p. 2460.

(24) KEPPELER, G., and SPAGENBERG, A.
1907. Notice on the flocculating action of colloids on clay suspensions. *In* Jour. Landw., Bd. 55, p. 299-300; Chem. Abs., v. 2 (1908), p. 1025.

(25) LINDER, S. E., and PICTON, H.
1895. Solution and pseudo-solution. Part II. Some physical properties of arsenious sulphide and other solutions. *In* Jour. Chem. Soc., v. 67, p. 63.

(26) LIPMAN, C. B., and WAYNICK, D. D.
1916. A detailed study of effects of climate on important properties of soils. *In* Soil Sci., v. 1, p. 5-48.

(27) LYON, T. L., FIPPIN, E. D., and BUCKMAN, H. O.
1915. Soils: Their Properties and Management, p. 159.

(28) MCGEORGE, W.
1915. Effect of fertilizers on the physical properties of Hawaiian soils. Hawaii Agr. Exp. Sta. Bul. 38, p. 19-22.

(29) MANSCHHAUPT, J. G.
1913. Remarks on Rohland's "The action of hydroxyl ions on clays and clay soils in marling." *In* Landw. Vers. Stat., Bd. 83, p. 467-470.

(30) MAYER, A.
1879. Uber die Einwirkung von Salzlösungen auf Absetzungsverhältnisse toniger Erden. *In* Forsch. Geb. Agr. Phys., Bd. 2, p. 251-273. Cited by Mitscherlich, Bodenkunde f. Land- u. Forst wirte (1913), p. 102.

(31) NORDENSON, H.
1915. Die Bedeutung des Lichtes für die Stabilität kolloider Lösungen. *In* Ztschr. Phys. Chem., Bd. 90, p. 603-628.

(32) ODEN, S.
1914. Colloid chemistry of humus. *In* Kolloid-Ztschr., Bd. 14, p. 123-30; Chem. Abs., v. 8 (1914), p. 2768.
(33) OSTWALD, Wo.
1909. Grundriss der Kolloidchemie, p. 508.
(34) QUINCKE, G.
1901. The clearing of turbid solutions and the movement of small suspended particles by the influence of light. *In* Chem. News, v. 84, p. 174.
(35) SCHLOESING, CH.
1870. Sur la précipitation des limons par des solutions salines très-étendus. *In* Compt. Rend. Acad. Sci. [Paris], t. 70, p. 1345-1348. Cited by Quincke (see Reference No. 34).
(36) SCHULZE, FRANZ
1866. Die Sedimentär-Erscheinungen und ihr Zusammenhang mit verwandten physikalischen Verhältnissen. *In* Ann. Phys. u. Chem. Hrsg. Poggendorff, Bd. 129, p. 366-383. Cited by Quincke (see Reference No. 34).
(37) [1]SCHULZE, —.
1882. ———. Cited by Kassuto, in "General Colloidal Chemistry (Russian Edition) p. 101.
(38) SOBRERO, A., and SELMI, F.
1850. Sur l'es produits de la décomposition des acides sulfhydrique et sulfureux au sein de l'eau. *In* Ann. Chim. et Phys., s. 3, t. 28, p. 210-214. An extract from a memoir read before the Royal Academy of Turin. Reale Accad. Sci. Torino Mem., v. 11, 1851, p. 407-412. Cited by Taylor, in "Chemistry of Colloids," p. 95.
(39) SPRING, W.
1900. Sur la floculation des milieux troubles. *In* Rec. Trav. Chim. Pays-bas et Belg., t. 19, p. 204-233. Cited by Kassuto in "General Colloidal Chemistry (Russian Edition) p. 116.
(40) TEAGUE, O., and BUXTON, B. H.
1908. Mutual coagulation of colloids. *In* Ztschr. Phys. Chem., Bd. 62, p. 287-307.
(41) TIEBACKX, F. W.
1911. Simultaneous coagulation of two colloids. *In* Ztschr. Chem. Indus. Kolloide, Bd. 8, p. 198-201; Chem. Abs., v. 5 (1911), p. 3753.
(42) VON-VEIMARNA, P. P. i ALEKSIEVA, A. V.
1914. Reactions taking place when dispersoid solutions are shaken with insoluble liquids or solids. *In* Zhurnal russkago fizikokhimicheskago obshchestva pri Imperatorskom S.- Petersburgskom universitetie. chast khimicheskaia. t. 46, vypusk 1., 133-135. St. Petersb.; Chem. Abs., v. 8, p. 2093.
(43) WHITNEY, M.
1891. Soil investigations. *In* Md. Agr. Exp. Sta. 4th Ann. Rpt., p. 258.

[1] Full reference not available.

INDEX

PAGE

Acid insoluble matter in loess soils.... 422-423
Acid Soils, Some Factors that Influence Nitrate Formation in (paper), E. B. Fred and E. J. Graul. See Nitrate formation in acid soils 317-338
Acidity—
  accumulation of due to sulfofication. 537-539
  increase of due to grinding..... 96-98
  of the medium as affecting enzyme activities of bacteria..... 192
Actinomyces—
  alboatrus 117, 127, 129
  alboflavus 120, 128, 129, 131
  albosporeus ... 121, 128, 129, 131
  albus 102, 117, 127, 129, 131
  aureus 124, 128, 129
  Bobili 121-122, 128, 129
  Californicus 122, 128, 130
  chromogenus group 102, 109, 114-115, 121, 127, 131, 132
  citreus 108, 118, 127, 129, 131
  diastaticus 116, 127, 129
  diastato-chromogenus 113, 127, 129
  erithro-chromogenus 112, 127, 130, 131
  exfoliatus 116, 127, 130
  flavus 118-119, 127, 130, 131
  Fradii 125, 128, 129
  griseus 119-120, 128, 129, 131
  Halstedii 124-125, 128, 129
  lavendulae 126, 128, 130
  Lipmanii 123, 128, 129, 131
  parvus 119, 128, 130
  purpeo-chromogenus 126, 128, 130
  purpurogenus 126, 128, 130
  reticuli 118, 127, 129
  roseus 125-126, 128, 129
  Rutgersensis 123-124, 128, 129
  Verne 120-121, 128, 129
  violaceus-Caeseri 111, 127, 129, 131
  violaceus-niger 111-112, 127, 129
  violaceus-ruber 110, 127, 130, 131
  virido-chromogenus 114, 127, 129
Actinomyces of the Soil, The (paper), S. A. Waksman and R. E. Curtis... 99-134
  ammonia accumulation by Actinomyces in the soil 131-132
  classification of the Actinomyces.... 109-110
  description of the Actinomyces..... 110-126
  key to the identification of the Actinomyces 129-130
  literature cited 133-134
  media used 103-104
  morphology of the Actinomyces..... 130-132
  numbers of Actinomyces in the soil, as related to bacterial numbers... 105-107

PAGE

  physiology of the Actinomyces..... 130-132
  salient features of the Actinomyces, tabular statement of..... 127-128
  soils used 103
Activity of protozoa in the soil..... 135-139
Albumen agar 104, 154, 155, 156, 157, 158, 159, 366
Alcohol (ethyl) used in soil sterilization 267, 273
Allison, F. E.—Brown, P. E., and (paper), The Influence of some Common Humus-Forming Materials of Narrow and of Wide Nitrogen-Carbon Ratio on Bacterial Activities. See Nitrogen-carbon ratio 49-75
Alumina—
  in loess soils 416-418
  iron, and, in the study of the effects of climate on soils 34-35
Alway, F. J., and Blish, M. J. (paper), The Loess Soils of the Nebraska Portion of the Transition Region: II. Humus, Humus-Nitrogen and Color. See Loess soils of the Nebraska portion of the transition region: II.... 239-258
Alway, F. J., and Isham, R. M. (paper), The Loess Soils of the Nebraska Portion of the Transition Region. III. Potash, Soda and Phosphoric acid. See Loess soils of the Nebraska portion of the transition region: III... 299-316
Alway, F. J., and McDole, G. R. (paper), The Loess Soils of the Nebraska Portion of the Transition Region: I. Hygroscopicity, Nitrogen and Organic Carbon. See Loess soils of the Nebraska portion of the transition region: I. 197-238
Alway, F. J., and Rost, C. O. (paper), The Loess Soils of the Nebraska Portion of the Transition Region: IV. Mechanical Composition and Inorganic Constituents. See Loess soils of the Nebraska portion of the transition region: IV 405-436
Ames, J. W., and Schollenberger, C. J. (paper), Accumulation of Salts in Ohio Soils 575-578
Amide nitrogen in soils and dried blood, 512, 515, 520-521, 524-526
Amide (acid) as acted upon by microorganisms 524-526
Amino acids, as acted upon by microorganisms 518

PAGE

Amino (mono) acid nitrogen in soils and dried blood 512, 515, 520-522
Ammonia—
accumulation—
as an index of bacterial and protozoan activity in the soil 143-149
by Actiomyces in the soil 131-132
evolved from soils 90-91
formation in the soil as an index of chemical changes in the protein molecule 516
nitrogen determination of, in the soil. 83
production, as a result of hydrolysis of proteins 517-519
Ammonification—
and nitrification in non-acid and acid soils 324-328
as affected by calcium carbonate.... 328-333
by Certain Soil Fungi, The Effect of Soil Reaction on (paper), N. Kopeloff. See Reaction, the effect of soil 541-573
by fungi, as affected by the biological stage of the organisms 279-283
experiments, in the study of the nitrogen-carbon ratio 54-62
studies, in the study of the effects of climate on soils 13-15
Ammonium sulfate, as used in nitrification 18, 19, 21, 63-64
Antiseptics—
relative sterilizing efficiency of various chemical substances 264-267
volatile, the relative efficiency of—
applied as vapor in partial vacuum 267-270
applied under heat and pressure.. 271-273
Arginine nitrogen in soils and dried blood 512, 515, 520-521, 523
Arid soils—
a Preliminary Statement as to the Status of the Humus Nitrogen Problem in (paper), C. B. Lipman. 285-290
compared with loess soils in Nebraska, 234, 256, 313-314, 433
Asparagine agar of Brown 154, 156, 157
Azofication experiments, in the study of the nitrogen-carbon ratio 65-70

Bacillus—
albidus 445-446
almus 446-448
cereus, enzyme activities of 187
cholera suis, enzyme activities of..... 187
coli, enzyme activities of 187, 188-193
concitatus 448-449
deciduosus 450-451
festinus 451-453
fluorescens liquefaciens 187
gilvus 453-455
immiutus 455-456
iugis 456-458
megaterium, enzyme activities of..... 187
proteus vulgaris, enzyme activities of... 187
subtilis, enzyme activities of..... 182-187
vulgaris, enzyme activities of..... 187
vulgatus, enzyme activities of..... 187

Bacteria— PAGE
action of cellulose-dissolving bacteria on the cellulose of plant tissues... 439-440
and actinomyces, numbers in the soil 105-107
cellulose-dissolving, characteristics, occurrence and activities 440-444
Diastase Activity and Invertase Activity of (paper), G. P. Koch. See Diastase activity 179-196
in Soils, Quantitative Media for the Estimation of (paper), R. C. Cook. See Media (quantitative) 153-161
occurrence of nitrifying bacteria in acid and non-acid soils 318-324
protein decomposition by, in its possible relation to enzyme activity.... 183-193
Bacterial—
Activities, The Influence of some common Humus-Forming Materials of Narrow and of Wide Nitrogen-Carbon Ratio on (paper), P. E. Brown and F. E. Allison. See Nitrogen-carbon ratio 49-75
counts, in the study of the effects of climate on soils 11-13
numbers—
and nitrate reduction affected by the addition of calcium carbonate 332-333
as affected by the—
presence of protozoa 141-150
use of antiseptics 268-273
in Soils, at Different Depths and in Different Seasons of the Year (paper), S. A. Waksman 363-380
carbon content of the soils 369
climatic conditions for the year.... 369
historical 363-366
lime requirement of the soils..... 369
literature cited 379-380
media used 366
methods employed 366-367
moisture content of the soils ... 370-375
nitrogen content of the soils..... 369
numbers of bacteria 370-377
soils used 367-368
summary 378

Bacterium—
castigatum 458-468
idoneum 460-461
lucrosum 461-463
mycoides—
ammonification by, as affected by the presence of protozoa..... 148-149
enzyme activities of 182-194
paludosum 463-465
Bacto-natural, used for the inoculation of soybeans 579-583
Baird, soybean, yield of dry matter and nitrogen content 175-178
Baryta in loess soils 420-421
Biological stage of fungi, as affecting ammonification 279-283
Black eyebrow, soybean, yield of dry matter and nitrogen content..... 175-178

PAGE

Blair, A. W.—
Lipman, J. G., and (paper)—
Factors Influencing the Protein Content of Soybeans. See Soybeans, factors influencing 171-178
The Yield and Nitrogen Content of Soybeans as Affected by Inoculation 579-584
and McLean, H. C. (paper), The Influence of Lime on the Yield and Nitrogen Content of Corn. See Lime, the influence on the yield.. 489-504
Blish, M. J.—Alway, F. J., and (paper), The Loess Soils of the Nebraska Portion of the Transition Region: II. Humus, Humus-Nitrogen and Color. See Loess soils of the Nebraska portion of the transition region: II.... 239-258
Blocks, soil, for the study of effects of climate 5-6
Blood, dried—
composition of its proteins 511-512
method of hydrolysis 512-513
used in nitrification 18, 20, 21
Bloodmeal agar 154, 155, 156, 157, 159
Bodo—
augustus, isolated from the soil..... 141
ovatus, isolated from the soil..... 141
Brown, P. E., and—
Allison, F. E. (paper), The Influence of Some Common Humus-Forming Materials of Narrow and of Wide Nitrogen-Carbon Ratio on Bacterial Activities. See Nitrogen-carbon ratio 49-75
Johnson, H. W. (paper), Studies in Sulfofication. See Sulfofication, studies in 339-362
Brown's—
albumen agar 154, 155, 156, 157, 366
asparagine agar 154, 155, 156, 157
casein agar 154, 156, 157
urea agar 154, 156, 157

Calcium carbonate, effect upon—
ammonification and nitrification, 322-324, 328-333
ammonification by soil fungi 556-571
bacterial numbers and nitrate reduction 332-333
sulfofication 358-359, 361
Carbohydrate decomposition by bacteria in its possible relation to enzyme activity 188-193
Carbon—
and Nitrogen Changes in the Soil Variously Treated: Soil Treatment with Lime, Ammonium Sulfate and Sodium Nitrate (paper), R. S. Potter and R. S. Snyder..... 76-94
ammonia nitrogen 83
apparatus, arrangement of 80-82
carbonates 84-90
historical 76-80
literature cited 93-94
methods of analysis 83-92

PAGE

nitrate nitrogen 84
nitrogen, total 83
summary and conclusions 92-93
treatment of soils 82-83
bisulfid used in soil sterilization.... 267-273
dioxide—
evolved from organic decomposition, composition of 89-90
given off per day in soil variously treated 86-88
in loess soils 414
nitrogen ratio in loess soils of Nebraska 229-231
organic—
humus ratio in loess soils of Nebraska 249-252
in loess soils of Nebraska 226-228
method of determination 226
tetrachloride used in soil sterilization, 272, 273
Carbonate content of soils 88
Carbonates, determination of in the soil. 84-90
Casein—
agar of, Brown 154, 156, 157
used for the study of ammonification and nitrification 320-330
used in ammonification experiments.. 54-57
Cellulose—
destruction, studies on, in the effects of climate on soils 28-30
dissolving bacteria—
action on the cellulose of plant tissues 439-440
occurrence and activity in southern California soils 440-444
from plants, method of preparation.. 439-440
in Soils, Studies on the Decomposition of (paper), I. G. McBeth.. 437-487
Bacillus—
albidus 445-446
almus 446-448
concitatus 448-449
deciduosus 450-451
festinus 451-453
gilvus 453-455
imminutus 455-456
iugis 456-458
bacteria, cellulose-dissolving—
action on the cellulose of plant tissues 439-440
characteristics, occurrence and activity 440-444
Bacterium—
castigatum 458-460
idoneum 460-461
lucrosum 461-463
paludosum 463-465
importance of cellulose destruction in soils 470-472, 477-479
introduction 437
key for identification 473-476
literature cited 481-487
media 437-439
plant cellulose, method of preparation 439-440

PAGE

Pseudomonas—
arguta 465-467
mira 468-470
minuscula 467-468
summary, general 479-481
summary of specific characters ... 470-472
medium, method of preparation 438-439
Chemical investigations, in the study of the effect of climate on soils..... 30-44
Chernozem—
soils compared with loess soils in Nebraska .. 231-234, 255, 313-314, 423, 432
types and conditions of formation in Russia 200-202
Chilodon, isolated from the soil..... 141
Chlamydomonas, isolated from the soil.. 141
Chloroform, used in soil sterilization, 267, 272, 273
Ciliates, numbers in the soil 140
Classification of the Actinomyces..... 109-110
Claud, soybean, yield of dry matter and nitrogen content 175-178
Climate, A Detailed Study of Effects of, on Important Properties of Soils (paper), C. B. Lipman and D. D. Waynick 5-48
alumina and iron 34-35
ammonification studies 13-15
blocks of soil used for the investigation 5-6
chemical investigations 30-44
cellulose destruction, studies on.... 28-30
coefficient, hygroscopic 8
colloidal content and volume of soils 10-11
color of soils 10
general theoretical and other considerations 44-46
humus 36
hygroscopic coefficient 8
iron and alumina 34-35
lime and magnesia 32-33, 43
literature cited 48
magnesia and lime 32-33, 43
manganese 34
moisture equivalent 9
nature and method of the investigation 7
nitrification studies 15-22
nitrogen, total, in the soil..... 37-38, 41
nitrogen-fixation studies 22-28
phosphoric acid 35, 41-42
potash 32, 42
reaction of the soils 38
silica, insoluble and soluble..... 30-32
soils, description of 7-8
sulfuric acid 35, 42
summary 47-48
water-extract studies 38-39
wilting point 10
Coefficient, hygroscopic 8-9
Coleman, D. A., Lint, H. C., and Kopeloff, N. (paper), Can Soil be Sterilized without Radical Alteration? See Sterilization, can soil be 259-274

PAGE

Colloidal—
content and volume of soils, in the study of effects of climate on soils 10-11
solution—
affected by—
muck colloidal solution 593
presence of solid material..... 593-595
effect of its concentration on the time required for coagulation... 595-596
efficiency of electrolytes in flocculating it 588-590
minimum electrolyte requirement for its flocculation 590-593
Colloids, Studies on Soil: I. Flocculation of Soil Colloidal Solutions (paper), M. I. Wolkoff 585-601
concentration of colloidal solution, effect on time required for coagulation 595-596
electrolyte, minimum requirement for the flocculation of a given amount of soil colloidal solution 590-593
electrolytes, efficiency of in flocculating soil colloidal solutions 588-590
introduction 585-587
literature cited 599-601
Mass Action Law applied to concentration of colloidal solutions by-electrolytes 595-598
methods 588
muck colloidal solution, effect on the stability of clay colloidal solutions.. 593
solid material present, effect on the stability of colloidal solutions..... 593-595
summary 597
Color of the loess soils of Nebraska... 253-255
Colorimetric method for the determination of humus 240-247
Colpidium colpoda isolated from the soil. 141
Colpoda cucullis isolated from the soil.. 141
Composting, as a means of providing a congenial environment for sulfofying bacteria 533-539
Concentration of colloidal solution, effect on time required for coagulation 595-596
Conn's sodium asparaginate agar 154-158
Contra-enzyme activities of microörganisms 185, 188, 191
Cook, R. C.—
(paper)—
Effect of Grinding on the Lime Requirement of Soils 95-98
Quantitative Media for the Estimation of Bacteria in Soils..... 153-161
Waksman, S. A., and (paper), Incubation Studies with Soil Fungi. See Incubation studies with soil fungi. 275-284
Corn, The Influence of Lime on the Yield and Nitrogen Content of (paper), A. W. Blair and H. C. McLean. See Lime, the influence on the yield. 489-504
Cottonseed meal, as used in nitrification 17, 19, 20

PAGE

Cotyledons, color of, as a criterion of toxic action of salts on soybeans..... 169
Cowpea soil, used for inoculation of soybeans 579-583
Crop yields, in the study of the nitrogen-carbon ratio 70-73
Curtis, R. E.—Waksman, S. A., and (paper), The Actinomyces of the Soil. See Actinomyces of the soil 99-134
Czapek's solution agar 104

Detrimental effect of protozoa upon bacteria 145, 147, 151
Diastase Activity and Invertase Activity of Bacteria (paper), G. P. Koch.. 179-196
diastase activity, method for determination of 181
enzyme activities of bacteria and the possible relation to their ability to decompose proteins 185-188
enzymes, secretion of, by bacteria in culture solutions 181-183
extra-cellular enzyme activity in a five-day incubation period 182-183
historical review 179-180
invertase activity, method for determination of 181-182
literature cited 194-196
protein—
and carbohydrate decomposition by bacteria in its possible relation to enzyme activity 188-193
decomposition by bacteria and its possible relation to enzyme activity 183-185
summary 193-194
Dilution method, used for the counting of protozoa 139-140
Dry matter, yield, in limed and unlimed plots 494-499
Dry weight of soybeans as a criterion of toxic action of salts 165-170

Ebony, soybean, yield of dry matter and nitrogen content 175-178
Edna, soybean, yield of dry matter and nitrogen content 175-178
Effect of Grinding on the Lime Requirement of Soils (paper), R. C. Cook... 95-98
Egg-albumen agar, used for the counting of bacteria 104, 154, 155, 156, 157, 158, 159, 356
Electrolyte, minimum requirement for the flocculation of a given amount of soil colloidal solution 590-593
Electrolytes, efficiency of, in flocculating soil colloidal solutions 588-590
Enchelys pupa, isolated from the soil... 141
Enzyme activities of bacteria and the possible relation to their ability to decompose proteins 185-188
Enzymes secreted by bacteria—
extra-cellular acitvity of 182-183
in culture solutions 181-183
Ether, ethyl, used in soil sterilization. 267-273
Euglena viridis, isolated from the soil.. 141
Euplotes, isolated from the soil 141

PAGE

Extra-cellular activity of enzymes secreted by bacteria 182-183

Factors Influencing the Protein Content of Soybeans (paper), J. G. Lipman and A. W. Blair. See Soybeans, factors influencing 171-178
Farmogerm, used for inoculation of soybeans 579-583
Ferguson's Composite, used for inoculation of soybeans 579-583
Flagellates, activity and numbers in the soil 136-140
Flora, Soil, Preliminary Experiments on Some Effects of Leaching on the (paper), C. B. Lipman and L. W. Fowler. See Leaching, preliminary experiments 291-297
Fowler, L. W.—Lipman, C. B., and (paper), Preliminary Experiments on Some Effects of Leaching on the Soil Flora. See Leaching, preliminary experiments 291-297
Fred, E. B., and Graul, E. J. (paper), Some Factors that Influence Nitrate Formation in Acid Soils. See Nitrate formation in acid soils 317-338
Fungi—
Soil, Incubation Studies with (paper), S. A. Waksman and R. C. Cook. See Incubation studies with soil fungi. 275-284
The Effect of Soil Reaction on Ammonification by Certain Soil Fungi (paper), N. Kopeloff. See Reaction, the effect on soil 541-573
The Inoculation and Incubation of Soil Fungi (paper), N. Kopeloff. See Inoculation and incubation... 381-403

Gelatin, used for the study of ammonification and nitrification 329-333
Glaucoma, isolated from the soil 141
Graul, E. J.—Fred, E. B., and (paper), Some Factors that Influence Nitrate Formation in Acid Soils. See Nitrate formation in acid soils 317-338
Gravimetric method for the determination of humus 242-247
Grinding Effect of, on the Lime Requirement of Soils (paper), R. C. Cook 95-98
Guelph, soybean, yield of dry matter and nitrogen content 175-178
Gypsum, effect upon—
ammonification 351-353
crop yields 353-355
sulfofication 344-351, 357-358

Halteria, isolated from the soil 141
Hay infusion agar 154-159
Heat used in intermittent sterilization of soils 261-264
Hilgard, Eugene Woldemar (biographical sketch) 1
Hilgard's contribution to the effect of climate upon soil types .....

PAGE

Histidine nitrogen in soils and dried blood 512, 515, 520-521, 523
Hollybrook, soybean, yield of dry matter and nitrogen content 175-178
Humic materials, effect on the stability of clay colloidal solutions 593
Humus—
and humus nitrogen—
ammonium hydroxide and sodium hydroxide extractions compared 287-288
in loess soils of Nebraska 240-252
ash, in loess soils of Nebraska.... 244-247
definition of and methods of determination 240-242
-Forming, The Influence of Some Common Humus-Forming Materials of Narrow and of Wide Nitrogen-Carbon Ratio on Bacterial Activities (paper), P. E. Brown and F. E. Allison. See Nitrogen-carbon ratio 49-75
in the study of the effects of climate on soils 36
Nitrogen Problem in Arid Soils, A Preliminary Statement as to the Status of the (paper), C. B. Lipman 285-290
ratio of, to nitrogen 247-251
Hydrochloric acid, normal solution of, as affecting ammonification by soil fungi 543-556
Hydrogen peroxide used in soil sterilization 267, 273
Hydrolysis of proteins 515-517
Hydrolytic processes, the variation of... 184
Hygroscopic coefficient 8-9, 214
Hygroscopicity—
in loess soils of Nebraska 214-219
its relation to mechanical composition of loess soils 410-411

Incubation—
the Inoculation and, of Soil Fungi (paper), N. Kopeloff. See Inoculation and incubation 381-403
period—
as affecting the accumulation of ammonia by cultures of bacteria and protozoa 146-149
as affecting the activity of enzymes secreted by bacteria 184-185
comparison of three and five days, for the development of bacterial colonies 156
for tests of sulfofication 356-357
for the study of ammonification by soil fungi 276-278
Studies with Soil Fungi (paper), S. A. Waksman and R. C. Cook... 275-284
biological stage of fungi, as affecting ammonification 279-283
incubation period and moisture relationship 276-278
introduction 275
moisture relationship 276-278
summary 283-284

PAGE

Influence of—
Some Common Humus-Forming Materials of Narrow and of Wide Nitrogen-Carbon Ratio on Bacterial Activities, The (paper), P. E. Brown and F. E. Allison. See Nitrogen-Carbon ratio 49-75
Various Salts on the Growth of Soybeans, The (paper), J. W. Shive. See Soybeans, the influence of the various salts 163-170
Inoculation—
and Incubation of Soil Fungi, The (paper), N. Kopeloff 381-403
incubation studies 394-402
literature cited 402-403
methods of the study of inoculation 382-383
Penicillium sp., study of inoculation 383-384
Rhizopus nigricans, study of inoculation and incubation.. 385-387, 398-402
Rhizopus oryzae, study of inoculation 389-390
spores, numbers of in inoculum, affecting ammonification 391-392
summary 402
Zygorrhyncus Vuilleminii, study of inoculation and incubation, 387-388, 394-398
The Yield and Nitrogen Content of Soybeans as affected by (paper), J. G. Lipman and A. W. Blair.... 579-584
Intermittent sterilization of soil by dry heat 259-264
Introductory (editorial) 3-4
Invertase activity—
of Bacteria, Diastase Activity and (paper), G. P. Koch. See Diastase activity and 179-196
method for determination of 181-182
Iron—
and alumina, in the study of the effects of climate on soils 34-35
in loess soils 418-419
Isham, R. M.—Alway, F. J., and (paper), The Loess Soils of the Nebraska Portion of the Transition Region: III. Potash, Soda and Phosphoric Acid. See Loess soils of the Nebraska portion of the transition region: III... 299-316
Ito San, soybean, yield of dry matter and nitrogen content 175-178

Johnson, H. W.—Brown, P. E., and (paper), Studies on Sulfofication. See Sulfofication, studies on 339-362

Key—
for the identifying of bacteria which dissolve cellulose 473-476
to the identification of the Actinomyces 129-130
Koch, G. P. (paper), Diastase Activity and Invertase Activity of Bacteria. See Diastase activity and 179-196

PAGE

Kopeloff, N.—
(paper)—
The Effect of Soil Reaction on Ammonification by Certain Soil Fungi. See Reaction, the effect of soil 541-573
The Inoculation and Incubation of Soil Fungi. See Inoculation and incubation 381-403
Coleman, D. A., Lint, H. C., and (paper), Can Soil be Sterilized without Radical Alteration? See Sterilization, can soil be 259-274

Lathrop, E. C. (paper), Protein Decomposition in Soils. See Protein decomposition in soils 509-532
Leaching, Preliminary Experiments on Some Effects of, on the Soil Flora (paper), C. B. Lipman and L. W. Fowler 291-297
alkalies added 291
ammonification 292-293
concluding remarks 296-297
literature cited 297
nitrification 293-295
nitrogen-fixation, non-symbiotic 295-296
Lime—
and magnesia, in the study of the effects of climate on soils 32-33, 43
in loess soils 413
requirement of soils—
effect of grinding on (paper), R. C. Cook 95-98
from limed and unlimed plots..... 495
used for nitrate accumulation.... 335-336
The Influence of, on the Yield and Nitrogen Content of Corn (paper), A. W. Blair and H. C. McLean 489-504
crop of 1908 493
crop of 1913 494
dry matter, yield of 494-499
literature cited 504
nitrogen—
percentage in dry matter and recovered 496-497, 499-503
total, recovered 496-497, 500-501
summary 503
Lint, H. C.—
Coleman, D. A., Kopeloff, N., and (paper), Can Soil be Sterilized without Radical Alteration? See Sterilization, can soil be 259-274
Lipman, J. G., McLean, H. C., and (paper), The Oxidation of Sulfur in Soils as a Means of Increasing the Availability of Mineral Phosphates 533-539
Lipman and Brown's modified synthetic agar 154, 156, 157
Lipman, C. B.—
(paper), A Preliminary Statement on the Present Status of the Humus-Nitrogen Problem in Arid Soils.. 285-290

PAGE

and Fowler, L. W. (paper), Preliminary Experiments on Some Effects of Leaching on the Soil Flora. See Leaching, preliminary experiments 291-297
and Waynick, D. D. (paper), A Detailed Study of Effects of Climate on Important Properties of Soils. See Climate, a detailed study of... 5-48
Lipman, J. G.—
and Blair, A. W. (paper)—
Factors Influencing the Protein Content of Soybeans. See Soybeans, factors influencing 171-178
The Yield and Nitrogen Content of Soybeans as Affected by Inoculation 579-584
McLean, H. C., and Lint, H. C. (paper), The Oxidation of Sulfur in Soils as a Means of Increasing the Availability of Mineral Phosphates 533-539
Loess Soils of the Nebraska Portion of the Transition Region, The—
I. Hygroscopicity, Nitrogen and Organic Carbon (paper), F. J. Alway and G. R. McDole 197-238
arid soils, compared with the loess soils 234
carbon, organic 226-228
carbon-nitrogen ratio 229-231
Chernozem soils, compared with the loess soils 231-234
climate 206-214
hygroscopicity 214-219
introduction 197-202
literature cited 236-238
methods of sampling 202-206
nitrogen 219-226
summary 234-236
volatile matter 229, 232
water of constitution 229, 232
II. Humus, Humus-Nitrogen and Color (paper), F. J. Alway and M. J. Blish 239-258
arid soils compared with loess soils.. 256
carbon (organic)-humus ratio 249-252
Chernozem soils compared with loess soils 255
color of the soils 253-255
colorimetric method for the determination of humus 240-247
gravimetric method for the determination of humus 242-247
humus—
ratio to nitrogen 247-251
studies on 240-252
introduction 239
literature cited 257-258
nitrogen, percentage of in humus. 251-252
summary 256-257
III. Potash, Soda and Phosphoric Acid (paper), F. J. Alway and R. M. Isham 299-316
arid soils, compared with loess soils 313-314
Chernozem, compared with loess soils 313-314

PAGE
citric acid soluble portions 306-313
hydrochloric acid soluble portions. 303-304
introduction 299
literature cited 316
separates of soil, composition of.. 304-305
summary 314-316
total amounts present 300-302
IV. Mechanical Composition and Inorganic Constituents (paper), F. J. Alway and C. O. Rost..... 405-436
acid-insoluble matter 422-423
alumina 416-418
arid soils, compared with loess soils. 433
baryta 420-421
carbon dioxide 414-415
chemical analysis of loess soils.... 412-431
Chernozem, compared with loess soils 423, 432
hygroscopicity, its relation to mechanical composition 410-411
introduction 405
iron 418-419
lime 413
litmus reaction 422
magnesia 415-416
manganese 420-421
mechanical analysis 406-410
literature cited 435-436
silica 419
sulfur 419-420
summary 434-435
titanium 421-422
water-soluble material 421
Lysine nitrogen in soils and dried blood. 512, 515, 520-523

McBeth, I. G. (paper), Studies in the Decomposition of Cellulose in Soils. See Cellulose in soils, studies on.... 437-487
McDole, G. R.—Alway, F. J., and (paper), The Loess Soils of the Nebraska Portion of the Transition Region: I. Hygroscopicity, Nitrogen and Organic Carbon. See Loess soils of the Nebraska portion of the transition region: I 197-238
McLean, H. C.—
Blair, A. W., and (paper), The Influence of Lime on the Yield and Nitrogen Content of Corn. See Lime, the influence on the yield 498-504
Lint, H. C.—Lipman, J. G., and (paper), The Oxidation of Sulfur in Soils as a Means of Increasing the Availability of Mineral Phosphates 533-539
Magnesia—
in loess soils 415-416
lime and, in the study of the effects of climate on soils 32-33, 43
Magnesium carbonate, effect on sulfofication 359-361
Manchu, soybean, yield of dry matter and nitrogen content 175-178
Manganese—
in loess soils 420-421
in the study of the effects of climate on soils 34

PAGE
Manhattan, soybean, yield of dry matter and nitrogen content 175-178
Mass Action Law, applied to the flocculation of soil colloidal solutions by electrolytes 595-598
Mechanical analysis of loess soils..... 406-410
Media for the Estimation of Bacteria in soils, Quantitative (paper), R. C. Cook 153-161
bacterial numbers in air-dry soil on the different media 157-158
comparison of three and five-day incubation periods 156
composition of media 154
literature cited 159
separate determination of bacterial numbers 160-161
summary 159
Medium Yellow, soybean, yield of dry matter and nitrogen content..... 175-178
Melanin nitrogen in soils and dried blood 512, 515, 520
Methods—
for the study of inoculation with soil fungi 382-383
of taking soil samples for the counting of bacteria 367
Microörganisms—
action on—
acid amides 524-526
amino acids 518
decomposition of proteins by 517
Modified synthetic agar, Lipman and Brown 154, 156, 157
Moisture—
as influencing the activity of protozoa and bacteria 143-150
content of the soil, as affecting the activity of protozoa 136-137
equivalent, in the study of the effects of climate on soils 9
relationship of soil fungi 276-278
Monas—
guttula, isolated from the soil 141
vivipara, isolated from the soil 141
Monilia sitophila, ammonification studies, 280-284
Muck colloidal solution, effect on the stability of clay colloidal solution..... 593
Mucor plumbeus, ammonfication studies, 275-284

Nassula elgeans, isolated from the soil.. 141
Nebraska, The Loess Soils of the Portion of the Transition Region—
I. Hygroscopicity, Nitrogen and Organic Carbon (paper), F. J. Alway and G. R. McDole. See Loess soils of the Nebraska portion of the transition region: I 197-238
II. Humus, Humus-Nitrogen and Color (paper), F. J. Alway and M. J. Blish. See Loess soils of the Nebraska portion of the transition region: II 239-258

PAGE

III. Potash, Soda and Phosphoric Acid (paper), F. J. Alway and R. M. Isham. See Loess soils of the Nebraska portion of the transition region: III 299-316
IV. Mechanical Composition and Inorganic Constituents (paper), F. J. Alway and C. O. Rost. See Loess soils of the Nebraska portion of the transition region: IV 405-436
Nitragin, used for inoculation of soybeans 579-583
Nitrate—
accumulation, effect on reaction..... 335-336
Formation in Acid Soils, Some Factors that Influence the (paper), E. B. Fred and E. J. Graul..... 317-338
ammonification in non-acid and acid soils 324-328
bacteria, nitrifying, occurrence in non-acid and acid soils 318-324
calcium carbonate, effect upon am-
calcium carbonate, effect upon—
ammonification and nitrification, 322-324, 328-333
numbers of bacteria and nitrate reduction 332-333
literature cited 337-338
nitrates, accumulation of in various soils 333-336
nitrification in non-acid and acid soils 324-328
reaction, affected by nitrate accumulation 335-336
soils used 317-318
summary 336-337
nitrogen, determination of in the soil. 84
reduction and numbers of bacteria affected by calcium carbonate..... 332-333
Nitrates, accumulation of in various soils 333-336
Nitrification—
and ammonification in non-acid and acid soils 324-328
as affected by calcium carbonate.... 328-333
experiments, in the study of the nitrogen-carbon ratio 62-65
studies, in the study of the effect of climate on soils 15-22
Nitrifying bacteria, occurrence in acid and non-acid soils 318-324
Nitrogen—
Carbon Ratio, the Influence of Some Common Humus-Forming Materials of Narrow and of Wide, on Bacterial Activities (paper), P. E. Brown and F. E. Allison 49-75
azofication experiments 65-70
blood, dried, ammonification of..... 57-62
casein, ammonification of 54-57
crop yields 70-73
effect of the materials added..... 52-54
literature cited 75
nitrification experiments 62-65
plan of the experiment 51-52
summary 73-75

PAGE

content of soils, determination of.... 92
fixation studies, in the study of the effects of climate on soils 22-28
forms of in dried blood and soil..... 512
hydrolizable, in soils 521
in loess soils of Nebraska 219-226
in the soil as ammonia and nitrate... 91-92
methods of determination of different forms in the soil 514-515
non-amino, in dried blood and soils, 512, 515, 520
percentage of, in humus in loess soils of Nebraska 251-252
recovered in corn, as affected by liming 496-497, 500-503
total, in the soil 37-38, 41, 83
Nitrogerm, Mulford and Standard, used for inoculation of soybeans 579-583
Nodule formation on soybeans, depression of 172-174
Numbers—
Bacterial, in Soils, at Different Depths and in Different Seasons of the Year (paper), S. A. Waksman. See Bacterial numbers in soils 363-380
of Actinomyces in the soil, as related to bacterial numbers 105-107
of bacteria, as affected by the presence of protozoa 141-150
of protozoa in the soil 140

Ohio—
9035, soybean, yield of dry matter and nitrogen content 175-178
Soils, Accumulation of Salts in (paper), J. W. Ames and C. J. Schollenberger 575-578
Organic—
carbon in loess soils of Nebraska.... 226-228
matter influencing activity of protozoa 138-139
Osmic acid, used in soil sterilization.. 269-273
Oxidation of sulfur in soils 533-539
Oxytricha, isolated from the soil 141

Paramoecium, isolated from the soil.... 141
Penicillium sp.—
ammonification of, in the study of inoculation 383-384
studies on incubation and moisture requirements 275-284
the effect of reaction on.... 552-554, 557-560
Peptone agar of Temple 154-157
Phosphate—
acid, effect upon—
ammonification 351-353
crop yields 353-355
sulfofication 344-351
mono-calcium, effect upon—
ammonification 351-353
crop yields 353-355
sulfofication 344-351
rock, effect upon—
ammonification 351-353
crop yields 353-355
sulfofication 344-351

PAGE
Phosphates, accumulation of available, in the soil as affected by sulfofication.. 535-539
Phosphoric acid—
in the study of the effects of climate on soils 35, 41-42
potash and soda in loess soils of Nebraska 299-316
Phyllomitus undulans, isolated from the soil 141
Physiology of the Actinomyces 130-132
Plant cellulose, method of preparation. 439-440
Pleuromena, isolated from the soil 141
Potash—
in the study of the effects of climate on soils 32, 42
soda and phosphoric acid in loess soils of Nebraska 299-316
Potter, R. S., and Snyder, R. S. (paper), Carbon and Nitrogen Changes in the Soil Variously Treated: Soil Treated with Lime, Ammonium Sulfate and Sodium Nitrate. See Carbon and nitrogen changes 76-94
Prorodon ovum, isolated from the soil... 141
Protein—
and carbohydrate decomposition by bacteria in its possible relation to enzyme activity 188-193
Content of Soybeans, Factors Influencing the (paper), J. G. Lipman and A. W. Blair. See Soybeans, factors influencing 171-178
Decomposition in Soils (paper), E. C. Lathrop 509-532
amide nitrogen in soils, 512, 515, 520-521, 524-526
amino (mono) acid nitrogen, 512, 515, 520-522
ammonia production 517-519
analytical results 514-515
arginine nitrogen in soils, 512, 515, 520-521, 523
dried blood 511-512
histidine nitrogen... 512, 515, 520-521, 523
hydrolysis of—
proteins 515-517
the soil and dried blood 512-513
hydrolyzable nitrogen 521
introduction 509, 510
literature cited 529-532
lysine nitrogen 512, 515, 520-523
melanin in soils 512, 515, 520, 526
nitrogen—
forms of, in dried blood and in soil 512
non-amino, in soils .. 512, 515, 520, 526
proteins in the soil at the end of the experiment 527-528
soils used 512
summary 528-529
Van Slyke's analysis applied to soils, 514-515, 519-520
molecule, structure of 516
Protozoa, Studies on Soil (paper), S. A. Waksman 135-152
activity of protozoa in the soil 135-139
ammonification affected by presence of protozoa 141-150
bacterial numbers, affected by protozoa 141-150
literature cited 151-152
numbers of protozoa in the soil 130-140
summary 150-151
types of protozoa in the soil 140-141
Pseudomonas—
arguta 465-467
minuscula 467-468
mira 468-470

Quantitative Media for the Estimation of Bacteria in Soils (paper), R. C. Cook. See Media (quantitative)... 153-161

Reaction—
of the soils, in the study of the effects of climate on soils 38
The Effect of Soil, on Ammonification by Certain Soil Fungi (paper), N. Kopeloff 541-573
calcium carbonate, used for change of reaction 556-573
hydrochloric acid, used for change of reaction 543-556
introduction 541-542
literature cited 571-573
methods 542-543
Penicillium sp., the effect of reaction on 552-554, 557-560
reaction altered by additions of—
$CaCO_3$ and H_2SO_4 556-573
normal solutions of HCl and NaOH 543-556
Rhizopus nigricans, the effect of reaction on 545-550, 565-570
sodium hydroxid, used for change of reaction 543-556
sulfuric acid, used for change of reaction 556-573
summary 556, 571
Zygorrhyncus Vuilleminii, the effects of reaction on ... 550-552, 560-565
to litmus, of loess soils 422
Rhizopus—
nigricans—
ammonification of, in the study of inoculation and incubation, 385-387, 398-402
the effect of reaction on.. 545-550, 565-570
oryzae, ammonification of, in the study of inoculation 389-390
Rost, C. O.—Alway, F. J., and (paper), The Loess Soils of the Nebraska Portion of the Transition Region: IV. Mechanical Composition and Inorganic Constituents. See Loess soils of the Nebraska portion of the transition region: IV 405-436

Salient features of Actinomyces, tabular statement of 127-128

PAGE

Salts—
Accumulation of, in Ohio Soils (paper), J. W. Ames and C. J. Schollenberger 575-578
The Influence of Various, on the Growth of Soybeans (paper), J. W. Shive. See Soybeans, the influence of various salts 163-170
Schollenberger, C. J.—Ames, J. W., and (paper), Accumulation of Salts in Ohio Soils 575-578
Schulze's valency law, applied to soil colloidal solutions 592-593
Shive, J. W. (paper), The Influences of Various Salts on the Growth of Soy-
Shive, J. W. (paper), The Influence of various salts 163-170
Silica—
in loess soils 419
insoluble and soluble, in the study of the effects of climate on soils..... 30-32
Snyder, R. S.—Potter, R. S., and (paper), Carbon and Nitrogen Changes in the Soil Variously Treated: Soil Treated with Lime, Ammonium Sulfate and Sodium Nitrate. See Carbon and nitrogen changes 76-94
Soda, potash and phosphoric acid in loess soils of Nebraska 299-316
Sodium—
asparaginate agar of Conn 154-159
hydroxide, normal solutions of, as affecting ammonification by soil fungi, 543-556
Soil Protozoa, Studies on (paper), S. A. Waksman. See Protozoa, studies on. 135-152
Soil's own nitrogen, as used in nitrification 17, 18, 20
Solid material, effect of on the stability of colloidal solutions 593-595
Soybeans—
a comparison of varieties 174-178
Factors Influencing the Protein Content of (paper), J. G. Lipman and A. W. Blair 171-178
nodule formation, depression of.. 172-174
rate of seeding 171-172
varieties used 174-178
variety tests in field experiments. 176-178
yield of dry matter and nitrogen content 175-178
The Influence of Various Salts on the Growth of (paper), J. W. Shive. 163-170
cotyledons, color of, as a criterion of the toxic action of the salts... 169
method of harvesting 165
salts employed 163-164
seeds used 164
soybean tops, dry weight of..... 165
toxic action of the salts, dry weight of plants as a criterion 166-170
The Yield and Nitrogen Content of, as Affected by Inoculation (paper), J. G. Lipman and A. W. Blair.... 579-584
Spores of fungi, numbers of, affecting ammonification 391-393

PAGE

Sporogen, used for inoculation of soybeans 579-583
Sterilization—
Can Soil be Sterilized without Radical Alteration? (paper), D. A. Coleman, H. C. Lint and N. Kopeloff 259-274
antiseptics, relative sterilizing efficiency 264-267
intermittent sterilization of soil by dry heat 259-264
literature cited 274
summary 273
vacuum chamber 265
volatile antiseptics—
applied as vapor in partial vacuum 267-270
applied under heat and pressure. 271-273
intermitten partial, as affecting the microörganic population of the soil 259-264
of soil, influencing the activity of protozoa 138-139
Strombidium, isolated from the soil..... 141
Studies on Soil Protozoa (paper), S. A. Waksman. See Protozoa, studies on. 135-152
Sulfates, amounts present in the soil.... 343
Sulfofication—
affecting the accumulation of available phosphoric acid 535-539
Studies in (paper), P. E. Brown and H. W. Johnson 339-362
ammonification tests 351-353
calcium carbonate, effect on sulfofication 358-361
conclusions 355, 362
crop yields 353-355
gypsum, effect on sulfofication.... 357-358
incubation period for tests of sulfofication 356-357
literature cited 362
magnesium carbonate, effect on sulfofication 359-361
method for determining sulfofying power of soils 339
plan of the experiment 340-342
sulfates present at sampling 342-344
sulfofication tests 344-351
Sulfofying power of soils, method for determining 339
Sulfur—
in loess soils 419-420
The Oxidation of, in Soils as a Means of Increasing the Availability of Mineral Phosphates (paper), J. G. Lipman, H. C. McLean and H. C. Lint 533-539
Sulfuric acid—
as affecting ammonification by soil fungi 556-573
in the study of the effects of climate on soils 35, 42
Swan, soybean, yield of dry matter and nitrogen content 175-178

PAGE

Tabular statement of salient features of Actinomyces 127-128
Tarheel, soybean, yield of dry matter and nitrogen content 175-178
Temple's peptone agar 154-157
Titanium in loess soils 421-422
Toluene, used in soil sterilization 267-273
Toxic action of salts on soybeans—
color of cotyledons, as a criterion of. 169
dry weight of plants, as a criterion of. 166-170

Urea—
agar of Brown 154, 156, 157
ammonium nitrate agar 154, 158, 159
Uroleptus, isolated from the soil 141
Uronema, isolated from the soil 141

Vacuum chamber for the sterilization of soil 265
Van Slyke's method for the partition of nitrogen, applied to soils... 514-515, 519-520
Varieties of soybeans, a comparison of. 174-178
Veitch method for determination of soil acidity, as affected by grinding 96-98
Volatile—
antiseptics applied—
as vapor in partial vacuum 267-270
under heat and pressure 271-273
matter in loess soils of Nebraska... 229, 232
Vorticella, isolated from the soil 141

Waksman, S. A.—
(paper)—
Bacterial Numbers in Soils, at Different Depths and in Different Seasons of the Year. See Bacterial numbers in soils 363-380

PAGE

Studies on Soil Protozoa. See Protozoa, studies on 135-152
and Cook, R. C. (paper), Incubation Studies with Soil Fungi. See Incubation studies with soil fungi... 275-284
and Curtis, R. E. (paper), The Actinomyces of the Soil. See Actinomyces of the soil 99-134

Water—
extract studies, in the study of the effects of climate on soils 38-39
of constitution in loess soils of Nebraska 229, 232
-soluble material in loess soils of Nebraska 421

Waynick, D. D.—Lipman, C. B., and (paper), A Detailed Study of Effects of Climate on Important Properties of Soils. See Climate, a detailed study of 5-48

Wilson, soybean, yield of dry matter and nitrogen content 175-178

Wilting point, in the study of the effects of climate on soils 10

Wolkoff, M. I. (paper), Studies on Soil Colloids: I. Flocculation of Soil Colloidal Solutions. See Colloids, studies on soil 585-601

Zygorrhyncus Vuilleminii—
ammonification of, in the study of inoculation and incubation, 387-388, 394-398
the effect of reaction on ... 550-552, 560-565